Genetic and Phenotypic diversity of cultivated Robusta coffee (*Coffea canephora* Pierre) in Uganda and effect of environmental factors on quality.

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This thesis is my original work and has not been presented for a degree in any other University or any other award.

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We confirm that the work reported in this thesis was carried out by the candidate. This thesis is submitted for examination with our approval as supervisors.

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Declaration

I confirm that this is my work and no part of this work has been submitted previously for a degree or examination in any other University.

I am responsible for the research and its articulation and in no way does any of the persons mentioned in the acknowledgement bear any responsibility.

This thesis does not contain other persons data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

DEDICATION

To my lovely children Paul Okiror, Aaron Oluka Mwaule, dear parents Aloysius Oluka (deceased) and Mary Lwisa Aguti Oluka for parental love, care, guidance and prayers. Uncle Father Augustine Opolot (deceased), thank you for shouldering the burden left by my late father with love; unselfishly you spiritually guided and materially provided.

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ABBREVIATIONS

µl	Micro litres
1CO	International Coffee Organization
A	Adenine
AFLP	Amplified Fragment Polymorphism
AMOVA	Analysis of molecular variance
ANOSIM	Multivariate analysis of Similarity
ANOVA	Analysis of variance
C	Cytocine
CGA	Chlorogenic acid
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CIP IV	Capital Improvement Programme 4
CIRAD	Centre for International Agricultural Research and Development
COREC	Coffee Research Centre
CV	Coefficient of variation
CWD	Coffee Wilt Disease
DATP	Deoxyadenosine triphosphate nucleoside
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate (dGTP)
DNA	Deoxy-ribonucleic acid
DTTP	Deoxythymidine triphosphate (dTTP)
EDTA	Ethylene Diamine Thiosulphate
EDXFR	Energy Dispersive X-Ray Florescence

EDXRF	Energy Disperse X-Ray Florescence
ELSD	Electronic Limited-Slip Differential (ELSD)
FADT	Factorial Analysis of Dissimilarity Table
FAO	Food and agricultre Organization, Italy
FID	Flame Ionization detection
FIS	Average Inbreeding Index
FIT	Inbreeding Index for Total samples
FST	Genetic differentiation
G	Guanine
GD	Genetic distance
GDP	Gross domestic product
GPC	Gel Permeation Chromatography
GPS	Geographical Positions Systems
H	Mahalanobis distance
H	Nei's unbiased estimate of gene diversity
Hcl	Hydrochloric acid
H_e	Expected heterozygosity
H_o	Observed heterozygosity
HPLC	High Performance Liquid Chromatography
HSD	Honestly significant difference
H_t	Total expected heterozygosity
IBPGR	International Plant Genetic Resources Institute, Italy.
ICO	International Coffee Organization

IF	Infra Red
IPGRI	International Plant Genetic Resources Institute
LSD	Least Significant Difference
m.c	Model coefficient
M.O.A	Memorandum of understanding
MATAB	Mixed Alkytrimetylammonium Bromide
MFED	Ministry of Finance, Planning and Economic Development
Mgcl2	Magnesium ch.loride
mM	Milli molar
MNHN	Museum National d' Historie Naturelle, France
MNR	Nuclear Magnetic Resonance
MS	Mean square
MS	Mass Spectrometry
MSc.	Masters of Science
N	Number of genotypes
Na	Number of different alleles
Ne	number of effective alleles
Ng	Nanogram
NIRS	Near Infra Red Spectroscopy
NJ	Un-weighted Neighbour Joining
Nm	Gene flow
Nm	Namometer
NMAED	Nutrient macro element density

ORSTOM	Presently IRD – Institut de recherché pour le d_eveloppement, France.
p.v	Probability value
Pa	Rare or private alleles
PAST	Palaeontological statistics 2.14 (PAST) programme
PC	Principle Component
PCA	Principle Component Analysis
PCI	Pressure Chemical Ionization
PCP	Principle Component Plot
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
Pmol	Parts per mol
ppm	Parts Per Million
PVPP	Polyvinylpolypyrrolidone
PWP	Precipitated Whey Protein
R^2	Coefficient of determination
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
Sdf	Significantly different factor
SIM	Selected Ion Monitoring
SSR	Simple Sequence Repeats selectively neutral markers
Std	Standard deviation
STS	Science, technology and society
T	Thymine

TLC	Thin Layer Chromatogrphy
U.K	United Kingdom
UCDA	Uganda Coffee Development Authority
UPGMA	Un-weighted Pair Group Arithmetic
UV	Ultra violet
v.r	Variance ratio

GENERAL ABSTRACT

Cultivated *Coffea canephora* in Uganda has low productivity with unknown quality trait variability. At present in Uganda, improvement of quality aspects of the *Coffea canephora* that would directly translate to increased incomes is hindered by the lack of information on the extent of the genetic and phenotypic diversity of the cultivated *C. canephora* and the effects of environmental influence on the coffee quality. The objective of this work was to evaluate the genetic and phenotypic diversity of cultivated *C. canephora* and assess the effects of the environmental influence on quality traits.

Genotypic variation, morphological and green bean physical characteristics, biochemical compounds and organoleptic cup attributes present in the local landraces were evaluated. The effects of environmental factors such as soil elements and texture, locations, altitude, rainfall and temperature at different bean development stages were also assessed.

In the experiments reported here, a total of 265 Robusta coffee genotypes collected from farmer fields in 13 traditional growing districts were analyzed using 32 Simple Sequence Repeats and were compared with 84 on station germplasm collections. SSRs efficiency and reliability was reflected by the low values for quality index and average minimum variance, detection of abundant and rare alleles to the extent of differentiation exemplified by allelic drift. Three genetic diversity groups with a mean genetic distance of 0.60 were different from those from Western and Central Africa possibly due to isolation or controls were few. Genetic diversity values were not antagonistic as all relationships were positive. High genetic variability among cultivated Robusta coffee

was demonstrated by the high Polymorphic Information Content (PIC) of 0.55 to 0.93 with a mean of 0.75 for 18 selected markers. The 54.05% within individual variability implied that genetic selection for desirable traits should be based on individual plants. Fairly polymorphic change over generations was revealed by the genetic differentiation value of 0.17 and gene flow mean value of 0.7±0.09 that caused exchange of one migrant between populations in each generation. The germplasm collection had fewer alleles, effective alleles, PIC, allele frequency and rare alleles as compared to accessions from farmer fields reflected limited variability among the few genotypes in the collection. Research controlled hybrid crosses and commercial varieties though genetically and morphologically, had limited genetic variability as each type confined to single groups.

Evaluation of morphological characters for 476 Ugandan farm Robusta coffee accessions showed that farmers cultivate predominantly 88% “nganda” and “erecta” landraces with improved types constituting 12%. Though ‘nganda’ and ‘erecta’ types were not genetically defined, were morphologically close and significantly different. The 206 Robusta coffee green bean physical characters evaluated using International Coffee Organization (ICO) criteria showed that 61.27% were of average screen size 15 and 19% larger beans.

The 16 accessions caffeine content analyzed using both Near Infra Red Spectroscopy (NIRS) and High Performance Liquid Chromatography (HPLC) methods were insignificantly different making NIRS technique better option because is fast and economically viable. Ugandan Robusta coffee biochemical compounds were aggregated

independently from that of CIRAD data base possibly because it was CIRAD's first encounter with Ugandan accessions and the possibly high species biochemical compound variability. The high fat (10.44 to 15.94 % dry matter) and chlorogenic acid (10.88 to 15.64 % dry matter) with a wide range of sucrose content (2.48-7.34% dm) indicated that Ugandan Robusta coffees are diverse with high fragrance, aroma and flavour. Other than potential to serve many markets, quality improvement and conservation purposes, the 4 multivariate groups were significantly different for dry matter and caffeine, implying it possible to select for dense beans with low caffeine to reduce bitterness in coffee brew. Genotypes in the three major genetic diversity groups distributed differently in the four multivariate green bean biochemical compounds diversity groups most likely due to the influence of environment on the latter. Lack of genetic diversity relationships with locations where they were grown meant that trait variations were strongly influenced by genotypes.

Evaluating roasted coffee green beans using the sensory technique developed by The Coffee Quality Institute of America (CQIA) alongside Uganda Coffee Development Authority, showed that the evaluator organoleptic cup trait rating was significantly different implying that individuals perceive cup taste differently. Positive correlation among organoleptic cup attributes implied that they complimented each other instead of antagonizing. Cup balance regression coefficient of 0.90 rated it as most important in overall cup evaluation. About 84% of Ugandan Robusta coffee brew was premium reflecting high quality. Acidity was least rated as average while other cup attribute rating ranged from premium to specialty grade pointing out that Robusta coffee can have as

good cup as Arabica coffee with medium acidity. Fragrance, aroma and flavour attributes could easily be selected from the 4 multivariate groups because because the attributes were significantly different. The present study has estimated optimum expression of different phenotypic quality traits at 30 to 35 years at a height of 1200 m.a.s.l. The genetic diversity and multivariate variability derived from this study offer numerous opportunities to select for breeding materials to develop diverse flavours that serve different markets, enrich existing germplasm collection, design further desirable trait studies and advice policy makers and coffee industry stakeholders. Cultivating at 1201-1300 m above sea level, use of “nganda” and “erecta” landraces, selecting trees of 31-40 years are some of the genotypic and environmental management practices that could be used to improve cup acidity.

Soil, leaf and green bean essential elements were detected and quantified using Energy Disperse X-Ray Florescence. Calcium, manganese, iron, zinc and lead mean content were higher in the soil and lowest in the green beans implying that not all the soil elements were taken up by plant. Potassium content in coffee was highest in the green bean because of its involvement in the metabolism and translocation of compounds such as oil, proteins and carbohydrates that influence cup quality. Although calcium is known to influence fruit ripening and quality, its content was highest in the leaves possibly because it’s a major constituent of cell walls and its content, involved in crucial functions such as cell division, root and leaf development, various enzymatic functions and transport carbohydrates.

Multivariate analysis of the effects of phenotype and environmental factors on quality traits revealed none of the variables was explained 100% by a single or a combination of phenotypic traits, elements, rainfall and temperature bean development stages suggesting that this trait is complex to select. Soil, leaf and bean elements explained most variability in organoleptic cup attributes (6.3 to 22.1%), bean physical characters (24.3 to 50.9%) and morphological characters (13.9 to 44.4%), possibly because elements are responsible for plant functions, vegetative development and constitute substances in cells based on genotypes. The highest chemical compound variability of 3.7 to 35.5% that was explained by rainfall and temperature bean development stages may have been a requirement for moderating plant function and facilitating access and movement of elements from the soil and in the plant. Most desirable bean size was average screen size 15 which more sucrose and less dry matter, caffeine and trigonelline content with better aftertaste, acidity, flavour.

This thesis evaluated cultivated *C. canephora* from traditional growing areas for genetic and phenotypic variability as well as influence of some environmental factors on quality traits. Information that has been derived is important not only in the identification of needed future research goals but the analysis of such diversity also goes a long way to contributing to an improved national economy and to raising the living standards of small-scale farmers of Uganda. Although all methods that have been used to assess quality traits are important, in case of meager resources and limited time, genetic diversity studies on quality can be complemented with fast, easy to use NIRS technique validated by organoleptic cup assessment which determines acceptability.

CHAPTER ONE: GENERAL INTRODUCTION

1.1. Background

As an export orientated commodity, coffee plays key role in national economies of nearly 80 developing world countries. About 25 million families supporting over 125 million people in more than 50 developing countries of Africa, Latin America and Asia produce and sell coffee as their main source of income (Aga et al., 2003). *Coffea canephora* Pierre ex Froehn represents 30% of the commercial coffee of the world and 80% of African production, of which Uganda is ranked among the top most producers (Orozco-Castillo et al., 1994). About 80% of the total Ugandan coffee production is Robusta estimated at 270,000 hectares while the 20% constitutes 40,000 hectares of *Coffea arabica* L. (UCDA, 2002; 2003). Uganda's agriculture sector depends mainly on coffee as an export crop while 8 million rural smallholder farmers involved in coffee cultivation and its enterprises rely on it as an important source of income (UCDA, 2001; 2002; MFED 2007).

Coffee generates over US $12.44 billion annually to the coffee producing and exporting countries of the world, with retail values of about US $70 billion (Osario, 2008). A study conducted on wild Arabica coffee of Ethiopia estimated the value of genetic resources to be between US $420 million up to US $1.45 billion while considering resistance to major coffee pests and diseases, low caffeine content and high yield (Hein et al., 2006). If other beneficial characteristics in wild coffees such as tolerance to drought were considered the value could have been much higher. In UCDA fiscal year 2007; 2008, coffee contributed 25% of the foreign currency earnings to Ugandan economy with the

export of 3.2 million 60-kilo bags of coffee being valued at US $ 388.4 million. An overview of the 2007 agricultural foreign currency earnings revealed that coffee exports earned US $ 265 million from sale of 2.52 million bags compared to fish at US $ 124 million, tobacco at US $ 66 million, tea at US $ 30 million and cotton at U$ 19 million (MFED, 2007). The highest foreign exchange from coffee was realized in 1996 when Uganda earned US $400 million from export of 4.2 million bags (UCDA 1996; 1997). Despite growth in other agricultural exports and continued decline in coffee volumes since 1997/98, coffee remains an important commodity to the economy and represents the major, frequently unique source of cash for smallholder farmers, enabling access to social, educational and medical services (Adipala et al., 2001; CWD survey report, 2002). With the present government's policy to promote coffee cultivation in non-traditional coffee growing areas, the acreage under Robusta is likely to increase (UCDA, 2007; 2008) and the population involved in its trade will also grow.

Coffee being a perennial cash crop provides regular income to farmers, and reduces food insecurity. It is estimated that in 2001/02 when coffee prices were at their lowest, farmers earned a minimum of Uganda Shillings 130,000 per acre (650 kg un-hulled coffee) spread throughout the year (UCDA, 2007; 2008). Because of the overall importance attached to coffee, it has continued to feature under various government programs such as PFA (Prosperity For All), NES (National Export Strategy), PMA (Plan for Modernization of Agriculture).

Farmers still cultivate *Coffea canephora* from cuttings or volunteer seedlings from natural populations (Leakey, 1970). Uganda cultivates two main types of Robusta coffee, distinctive enough to be called varieties, the erect form "erecta" (known as *Coffea quillou*) and the more spreading type locally known as "nganda" (*Coffea ugandae*). The "erecta" types tend to have strong stems, pale large leaves with bigger berries. The "nganda" types usually have weaker upright stems that tend to bend or break with heavy crop. The "nganda" leaves are typically small and dark green with yields that catch up with "erecta" yields at 4-5 years, the age at which "erecta", unless under good field management start declining and trees suffer die back due to over cropping (Thomas, 1940; Kibirige-Sebunya et al., 1993). The two forms are grown in mixtures and freely cross (Maitland, 1926; Butt et al., 1970; Thomas, 1935) making it difficult to have distinct "nganda" or "erecta" types. In 1910, an erect form of Robusta was introduced from Java (originating from Zaire and later enriched with materials from Gabon and Uganda) and planted mainly in European estates (Charrier et al., 1985; Thomas, 1935; 1944; 1947), but when evaluated, it yielded results that attracted no further interest.

The average Robusta coffee yields at farm level are about 600 kg clean coffee per hectare and constitute only 10-30% of what can be achieved potentially or what is commonly achieved in similar ecologies in Asia and Latin America (UCTF, 2008; 2009). With moderate crop husbandry, average yields of 2,500kg/ha can be realized (Kibirige-Sebunya et al., 1993). All coffee grown in Uganda is rain fed. Usually, coffee trees are planted at variable spacing, often intercropped, naturally lightly shaded and rarely mulched. Fertilizer, herbicides or pesticides are rarely applied. Co-existence of coffee with other

crop and shade species plays a significant role in the ecosystem conservation, survival capacity of the species, food security and alternative source of income. Hoes, slashers, pangas, and bow saws are rudimentary tools commonly used to clear weeds and manage tree canopy. Farm management is mainly by family labour (UCTF, 2008; 2009).

1.2. Problem Statement

Increasing coffee consumption and production is a challenge for improving Robusta competiveness though quality. Ugandan coffee production is 80% of Robusta which is regarded inferior in cup quality as compared to Arabica. Coffee quality is normally determined by seed, liquor and product (Charrier et al., 1985). Small bean size, lack of good flavor and pronounced bitterness in the beverage and inability to endure long drought periods are some of the undesirable traits in Robusta coffee (Wrigley, 1988; Prakash et al., 2005). Subsequently, the general market value for *C. canephora* has persistently remained lower than that of *C. arabica* in spite of the several useful traits endowed in *C. canephora* such as tolerance to disease like leaf rust pathogen, white stem borer and nematode invasion (Prakash et al., 2005). Even if Robusta coffee brew is rated neutral, with body and occasionally strength, some genotypes have cup quality that is as good as that of Arabica (Charrier et al., 1985), unfortunately without statistical documentation.

Frequent world coffee export price slump have drastic consequences on prices of coffee up to farm gate level especially for Robusta. For example, in 1999-2001, average farm gate prices for un-hulled Robusta (Kiboko) fell from Uganda shilling (UGSH) 647 to 200 UGSH per kilogram (UCDA, 2007; 2008). Average farm gate price for Arabica declined much more slowly from 1830 to 1200 Uganda shilling per kilogram of parchment,

reflecting the higher demand for higher quality coffees. In 2001, Uganda's best mild Arabica coffee was exported at twice the price of the highest Robusta (16 –18) (Ponte, 2002a).

Coffee research is mandated to conduct and conserve coffee plant genetic resources. To date research has concentrated mainly on technology generation and dissemination in diverse environments with minimal emphasis on quality trait improvement. To enhance Robusta coffee productivity and quality, keep up with environmental changes and coffee market demands, a variable genetic base is required to provide desired consumer traits. Strategic conservation also demands prior comprehensive knowledge of naturally existing genetic structure of wild and farm landraces. So far, the genetic diversity of wild Robusta coffee in Uganda has been classified into four groups (nganda and erecta, feral from Kalangala, Itwara and Kibale forests that constituted a group belonging to the Congolese SG2 subgroup in Central Africa. Phenotypic and heritability variations for coffee wilt disease, *Fusarium xylariodes* Steyaert observed among Ugandan C. *canephora* clones and progenies (Musoli et al., 2013) explain the potential variability endowed within the Congolese major group.

It is of uttermost importance to establish the genetic diversity and phenotypic variability of cultivated Robusta coffee quality traits and some important environmental factors that influence cup quality as a basis for quality improvement and conservation. Like wild and semi wild forms, Robusta coffee land races in the farmer fields are under continuous genetic erosion with no conservation strategy put in place. The emergence of Coffee Wilt Disease (CWD), caused by *Fusarium xylariodes* Steyaert, since 1993, has destroyed 44% of the 242, 000 ha of cultivated Robusta coffee countrywide. Profound global effect of

climatic change is taking toll of genetic resources in such a way that the less adapted will be eliminated though may have valuable agronomic traits. Furthermore, farmers are encouraged to replace their heterogeneous local landraces with the few improved varieties developed from a narrow genetic base. The discovery of oil is likely to affect some arable land and government's attention may divert from Agriculture in general and coffee in particular to oil mining and trade.

The collections at Kituza and Entebbe botanical gardens that were done in 1920's are few and are not properly documented. At present, there are limited labeled Robusta coffee accessions at Kawanda Research (Wrigley, 1888). The Kawanda germplasm collection underwent rigorous testing for yield, coffee leaf rust (*Hemilelia vastatrix*), red blister (*Cercospora coffeicola* Berk et Cooke) and liquor acceptability. Lack of information on genetic diversity has hampered the exploitation of the available gene pool in meeting the growing market demands and has also affected the conservation and utilization of the germplasm. In absence of any information on genetic diversity, any improvement endeavour is time consuming and expensive.

1.3. Justification

Coffee marketing is driven by the quality and health aspects of the final product. Promoting coffee quality is likely to add value, enhance income and competitiveness world coffee prices. In addition, it will lead to reliable markets and sustainable development of the coffee growing areas. Robusta coffee organoleptic cup characteristics, though different from those of Arabica coffee can be equal to those of Arabica when carefully selected and processed. In the past in Uganda, liquor improvement was not considered because the coffee that was produced had always been graded and sold

(Leakey, 1970; Charrier et al., 1985). In order to sustain coffee industry in Uganda, coffee quality improvement program has to be developed. This is only possible if cultivars with a superior organoleptic cup quality are selected existing variability that may include morphology and physical caharcters, physiological and green bean biochemical compound characteristics (Leroy et al., 2006). Equally, environmental factors such as soil texture, nutrient element composition, altitude, rainfall, temperature that directly or indirectly contribute to coffee quality need to be considered (Decazy et al., 2003; Geromel et al., 2008). Harvest, post harvest and processing procedures up to cup brew level are also important factors influencing cup quality.

To date, research has limited Robusta coffee germplasm base. National collections of the wild and cultivated Robusta coffee done in the early 20th century (Jameson, 1970; Maitland, 1926) were not representative of the coffee mother plants. Due to historical omissions and events, the original parental stock at Kawanda germplasm collection was lost (Thomas, 1935; 1944; 1947). There has also been a gradual sizable loss of trees to old age, pest and diseases without any addition of variability.

A genetic diversity study is fundamental as it reveals heterogeneous genetic resources with high evolutionary potential or alternative sources that control particular traits such as cup quality. As a consequence, the future survival of Robusta trade will specifically depend on research utilization of available information. Markets too are increasingly attaching price tags to coffee quality and appellations. To develop an effective Robusta coffee quality improvement strategy, it is important to first investigate the diversity of naturally existing cultivated *C. canephora* as a bench mark for sourcing additional variability. Information on morphological characters in genetic diversity help choose

parental materials for breeding program and provide basic information on existing plant morphology and genetic variability. As regards coffee quality assessment, additional information on green bean physical characters and biochemical compound trait variability influencing organoleptic cup are important.

1.4. Overall Objective

Assess factors that influence *C. canephora* market valued traits.

1.4.1 Specific Objectives

a. To characterize the molecular diversity of cultivated *C. canephora* in Uganda.

b. To evaluate the phenotypic variation of cultivated *C. canephora* in Uganda.

c. To assess the quality attributes of cultivated *C. canephora* in Uganda.

d. To determine the effect of phenotypic traits, micro and macro nutrients, rainfall and temperature on cultivated *C. canephora* quality traits.

1.5. Hypothesis

a. There is no genetic diversity among cultivated *C. canephora* genotypes.

b. Cultivated *C. canephora* morphological and green bean physical characters, biochemical compounds and organoleptic cup attributes are not variable.

c. There is no variability among soil, leaf and green bean nutrient element and soil nutrient elements are not components of *C. canephora* leaves and green beans.

d. Phenotypic and environmental factors do not influence the variability of quality traits in *C. canephora*.

CHAPTER TWO: LITERATURE REVIEW

2.1 Coffee origin and taxonomy

Coffea is a woody perennial dicotyledonous plant which is indigenous to Africa and is a major genus of the Rubiaceae family widely distributed in the tropics (Thomas, 1940). The genus *Coffea* L. is reported to have 105 species (Medina-Filho et al., 2007), which are prevalent to Africa and Madagascar (Prakash et al., 2005). The montane rain forests of Ethiopia on both western and eastern slopes of the Great Rift Valley and Boma plateau in the Sudan are the centres of origin of *C. arabica* (Maitland, 1926; Fernie, 1970; Wrigley, 1988). *C. canephora*, is a plant of equatorial Africa flourishing under conditions that are fairly humid throughout the year. Robusta coffee cultivation is most suitable between latitude 10^{o} N and 10^{o} S. *C. canephora* spreads from Guinea to Uganda and Sudan (West to East), and from Cameroon (North) to Angola (South) (Thomas, 1940; Berthaud, 1986; Leakey, 1970). *Coffea liberica* Bull. Ex. Hiern which is known for its resistance to diseases, insect pests, adaption to low elevation and hot temperature is native to the tropical forests of the present Liberia and probably the Cote de Ivoire (Prakash et al., 2005). The forests of Madagascar and Mascarene Islands (Mauritius and Reunion), are the home of *Coffea mascara*, a species, characterized by low levels or complete absence of caffeine.

Many diploid species in Africa overlap in origin (Thomas, 1940; Leakey, 1970; Berthaud, 1986). Wild coffee species found in Ugandan forests are *C. eugenioides* S. Moore, *C. excelsa* Chev and *C. spathicalyx* K. Schum. *C. canephora* although widespread with strong adaptations to rainfall and soil types occurs less frequently than *C. eugenioides* and grows naturally between 1000 and 2000 m above sea level in forests

located in Bunyoro, Buganda and in the North close to the Sudan boarder (Thomas, 1935; 1947; Leakey, 1970). The diverse existence of the genus *Coffea* in Uganda suggests that the country may be within the centre of origin (Maitland, 1926; Thomas, 1940; Leakey, 1970). Robusta coffee trees of 120-150 years have been observed in Ssese Islands, Uganda (Wrigley, 1988).

Whereas three of the genus *Coffea* species are economically important, coffee production and its industry depend on two species commonly known as Arabica coffee (*Coffea arabica* L.) and Robusta coffee (*Coffea canephora* Pierre ex Froehn) (Coste, 1992; Thomas, 1944). *C. arabica* constitutes 70% and *C. robusta* 30% of the total world production as well as export (Orozco-Castillo, 1994). *Coffea liberica* is the third economic species, produced mainly in Liberia, parts of Java, Malaysia and the Philippines but because of its low yield and poorer quality, is used only for local consumption. With advanced breeding techniques, commercial interspecific hybrids such as Arabusta (*C. arabica* X *C. canephora*) have been developed. However blending coffees from the two species at varying ratios probably produces the desired consumer flavours at minimal costs (Leroy et al., 2006).

Variation in chloroplast and mitochondrial DNA in the genus *Coffea* support the hypothesis that *C. canephora* diverged from *C. congensis* and there after the latter species differentiated into *C. eugenioides* and *C. arabica* (Berhou et al., 1983). Taxonomic grouping based on diterpene profiles indicate that *Coffea* species universally contain cafestol but Arabica coffee contain 'Kahweol' diterpene only in varying structures (Baukje de Roos et al., 1997). With exception of C. *arabica* that is tetraploid and self-fertile (2n = 4x = 44), all the other species in the genus *Coffea* are diploid (2n = 2x = 22),

with gametophytic self incompatibility and hence can exchange genetic material with cultivated *C. canephora* (Robusta) (Charrier et al., 1985; Ferwerda, 1954). Given its allopolyploidy and self-pollinating nature, *C. arabica* is characterized by low genetic diversity leading to a narrow genetic base (Aga, 2005).

2.2 Description of coffee tree characters

All *Coffea* species are evergreen, woody and range from shrubs to trees in size that can grow to a height of 10 meters. The stems are vertical (orthotropic). The horizontal (plagiotropic) branches (primaries) arise in pairs opposite each other and remain subsidiary to the main stem and develop sub lateral; secondary, tertiary and quaternary branching. Branches far away from main stem apex are larger. The leaves are shiny on the upper side, generally waxy, spear shaped and grow in pairs on either side of the stem or branch with buds at the leaf stalk base. Leaf colour range from yellowish to dark green with a greenish, bronzed or purple tinged young flush. Leaf length varies from 1-40 cm. Some trees are deciduous while others retain leaves for 3 or more years with a leaf area index of 7-8 for a high yielding variety (Wintgens, 2004). Some trees have fragrant flowers with 5-6 petals. Coffee pollen is not sticky, is light and carried by insects and wind up to a height of 8 m and a distance of 100 m (Leakey, 1970). Pollen can remain viable for over a month under special conditions, otherwise it loses viability after a few days (Wrigley, 1988). The vertical, tap and lateral root system grow parallel to the ground and variably extend to a depth of 90 cm with the tap root penetrating up to 30-45 cm. Lateral roots may extend 2 m from the trunk. 80-90% of the feeder roots remain in the 20 cm depth and 60-90 cm radius (Wrigley, 1988). The root system is influenced

by the soil type and the mineral content of the soil, as their thickness and strength require extensive supply of nitrogen, calcium and magnesium.

2.3 Conditions for coffee growth

2.3.1 Altitude and latitude

Coffea is a tropical plant that requires specific environmental conditions for commercial cultivation. Altitude, latitude, temperature, rainfall, soil, sunlight, wind and humidity influence the quality of the developing bean from flowering, bean expansion until ripening (Cannell, 1985, Clifford et al., 1985; Muschler, 2001; Decazy et al., 2003; Vaast et al., 2006;). Production of beans with superior beverage quality appears to be highly dependent on the climatic conditions (Decazy et al., 2003). At best, Arabica coffee grows at altitude range 1200-1800 meters above sea level. Arabica coffee can also be grown below 400 m above sea level between 25^{o} Tropic of Cancer in the North and 25^{o} Tropic of Capricorn in the South provided there is no frost. Robusta coffee cultivation is most suitable between latitude 0^{o} to 10^{0}. Robusta coffee can be grown between sea-level until such a height when vegetative growth with poor crop setting is reached. Gentle but not steep slopes to fairly level fields are ideal for Robusta coffee. Under Ugandan conditions where soil has less influence on the distribution of Robusta than climate (Wrigley, 1988), most Robusta coffee is cultivated in the range of 1000 to 1500 m above sea level. Nearer to the Equator, coffee plants require higher altitudes compared to farther North and South. Beyond latitudes 23^{0}N and 25^{0}S, temperature becomes a major limiting factor because Robusta coffee plants are more sensitive to extreme temperatures (Wrigley, 1988).

2.3.2 Temperature

It is generally accepted that 100 meters increase in elevation decreases mean daily temperature by 0.6–1^{0}C (Rojas, 1985). High temperatures can be harmful, especially if the air is dry (Coste, 1992). Suitable temperatures for plant growth change with the phenological stage of coffee (DaMatta et al., 2006). For example, an optimum temperature of about 30-32^{0}C over a 3 weeks period is adequate for Arabica coffee to accomplish the germination process while at 17^{0}C germination takes 3 months and at 35^{0}C germination is inhibited. Robusta coffee prefers warm temperature range of 24-30^{0}C but not below 15^{0}C. Temperatures of up to 30^{0}C are also needed for floral bud initiation. For adequate root development, the best soil temperature is 24-27^{0}C. It is also estimated that a 2^{o}C rise in temperature would affect coffee production in Uganda drastically, reducing the total coffee area by three quarters (UCTF, 2008; 2009).

2.3.3 Rainfall

The pattern of rainy and dry periods is important for growth, budding and flowering. Rainfall requirements depend on the retention properties of the soil, atmospheric humidity, cloud cover and cultivation practices. An optimum annual rainfall range of 1200-1800 mm is required for Arabica coffee while Robusta coffee adapts to intensive rainfall exceeding 2000 mm at a relative humidity of 80-90% and for a short while in less humid sites during dry season (Coste, 1992). In contrast, Arabica coffee requires a less humid atmosphere (Haarer, 1958; Coste, 1992). Air humidity has a significant impact on the vegetative growth of the coffee tree. For both species, a short dry spell, lasting 2-4 months corresponds to the dormant growth phase that stimulates flowering (Haarer, 1958). Lack of dry period can limit coffee cultivation. Meanwhile abundant rainfall

throughout the year causes scattered harvest and low yields, water deficiency results to empty and dry cherries. However, *C. canephora* variety Kouillou acclimatizes much better to drier environments than the *C. canephora*, variety Robusta.

Rainfall of about 2.5 cm received either on one day or in 2-3 consecutive days adequately induces normal blossom in a productive Robusta coffee tree. Unless irrigated during flowering period, poor flower bud growth and poor fruit set may result. Coffee requires bright and light winds on the day of flower opening for proper dispersal of pollen grains by wind and insects. Stormy, rainy, frost and hail storm damage flowers, cherries and other vegetative parts of coffee tree reducing green bean quality. Because of shallow root system and larger leaf area, Robusta coffee is more sensitive to moisture stress as compared to Arabica coffee. At the same time Robusta coffee does not perform well in very high rainfall regimes. Generally, coffee growing areas receive rainfall of 1500-2500 mm per annum. After fertilization, a well distributed rainfall of 1200 mm to 2000 mm over a 9 month period is ideal for Robusta coffee bean development (Cannell, 1985; De Castro et al., 2006).

Diverse environmental factors that include rainfall influence the 5 growth stage bean development period (Geromel et al., 2006). At pin head (6-8 weeks) that follows fertilization, backing showers of 2.5 to 4.0 cm are required within one month after blossom showers for the retention of set fruits or else they dry up reducing yield. Moisture content is important for rapid cell growth (10-15 weeks) of locules containing ovaries to develop into swollen drupes as drought constrains normal growth (Cannell, 1985). Water stress at 6-16 weeks decreases physiological activities of the developing coffee beans and can result to empty or smaller beans. The endosperm enlarges filling

and replacing the integument cavity with a silver skin at 12-19 weeks (Cannell, 1985; De Castro et al., 2006).

Water is required to generate 70% of photosynthetic products like storage proteins, sucrose and complex polysaccharides, used to harden the endosperm at full formation stalling plant growth. Finally at 30 to 35 weeks, the endosperm is dehydrated and the pericarp {exocarp (peel), mesocarp (sweet pulp is rich in reducing sugars, sucrose) and the endocarp} changes color to red or yellow (De Castro et al., 2006). A single dry period of 2-3 months that coincides with the harvest season is required for drying and for coffee trees to recover after harvest (DaMatta et al., 2007). However, Uganda being along the equator has two rainy and dry seasons, resulting in two cropping cycles per year. Main harvest season in the Northern hemisphere occur around November to January while that for the Southern hemisphere is April to June. In practice, any sporadic rain after a dry spell creates uneven flowering and fruit ripening in Robusta coffee.

2.3.4 Plant nutrient element requirement in coffee

Coffee roots require much oxygen that is inadequate in heavy, poorly drained and clay soils. Coffee grows on soils of widely different geological origin that include gneiss, sandstone, limestone, basalt, lava, alluvial and colluvial soils of volcanic ash texture with a high Base Exchange capacity (Wrigley, 1988; Zake et al., 1996; Wintgens, 2004). A free drainage depth of about 1.5 m to 3 m in drier areas permits the development of roots over wider ground coverage. A water table of lower than 1.5 meters suffocates the tree root systems with a flood condition. Though coffee requires deep (at least 75cm), rich organic matter content (>2.5 per cent organic carbon), friable, open textured and permeable soil that allow gaseous exchange and drainage, the crop can grow on soils of

different depths, texture neutral acidity (6.0-6.5 pH) and mineral content, given suitable applications of fertilizer (Wrigley, 1988).

For healthy Robusta coffee growth, development and production, coffee trees requires macro and micro nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), Magnesium (Mg) (Zake et al., 1996) in different quantities or combinations. About 90% NPK and Mg, 39% of Ca are taken by fruits where they are rapidly metabolized. Even if mycorrhiza fungi and nitrogen fixing bacteria may improve soil fertility, coffee is a heavy feeder crop that loses about 30% of soil nutrients to the harvested ripe cherries. To obtain one metric tonne of Robusta coffee green cherries requires 30kg of N, 3.8 kg P2O5 and 47.8 kg of K2O from soil (Onzima et al., 1996). Application of 500 g Ammonium Sulphate Nitrate (ASN) and 50 g Single Super Phosphate (SSP) fertilizer per tree per year (Zake et al., 1996) restores soil fertility for normal coffee growth. Coffee residues like husks, pulp, pruning, culls (rejects) and seed coat can be added as compost and mulch to coffee field to amend soil structure and nutrients (Kibirige-Sebunya et al., 1996). According to Zake et al., 1996, use of coffee husks provide high levels of potassium (4%), nitrogen (1.5%), considerable amounts of phosphorus and calcium with a high carbon and nitrogen ration of 5:1. Zake et al., 1996 further indicated Ugandan soils were acidic with high nitrogen, available phosphorus and low levels of exchangeable bases. With a high carbon and nitrogen ratio, chemical fertilizers are required to hasten mineralization and release of nutrients.

Trace element deficiencies of manganese, iron, boron and zinc usually can visually be detected by leaf chlorosis (leaf yellowing, sometimes in characteristic patterns), leaf whitening (purple, dark green, bronze discoloration) stunted growth, necrotic areas,

susceptibility to root rot, leaf curl and crinkle, bent stem (lodging) and premature abscission. Lime (CaCO3) is often applied to help improve acidic soils to a pH range of 4.5-5.5 in the first 20 cm of soil (Zake et al., 1996). Depleted soils can be improved with inorganic fertilizer with micronutrients especially in leached sandy acid soils. Organic fertilizers have to be mineralized to avail nutrients. Mycorrhizal fungi can be used to improve soil quality (Zake et al., 1996; Kibirige-Sebunya et al., 1996). Regions with low annual precipitation or long dry season normally have sandy, rocky and heavy laterite soils which have poor water retention and are not suitable for coffee growing. Suitable soils for coffee cultivation should contain no more than 20-30 % coarse sand (larger than 2mm in size) and 70 % of clay in the 30 cm upper layer. (Mitchell, 1988) Hard-pans of rock, lava, calcareous tufa or mud-stone origin are dense, thick and impenetrable by coffee roots. In cases where Robusta coffee has either by-passed or penetrated through fissures and clefts of laterite shield, trees grow well because there is ample provision for nutrients, air and water infiltration.

Acidity and organic matter content are the major factors that control soil nutrient supply. Organic matter promotes microbial activities that improve soil structure, aeration and water holding capacity allows soil to respond to inorganic fertilizers (Zake et al., 1996). Fresh manure is not recommended as it generates heat during decomposition that burn plants, holds nitrogen in the soil and is a source for Orhratoxin A (OTA) moulds. Mulching using dry grass and crop residue conserves soil moisture, protects soil from compaction, and reduces soil acidity (Onzima et al., 1996). Farm yard manure or increased exchangeable potassium improves nutrient uptake (Kibirige-Sebunya et al.,

1996). High potassium improves metabolism of nitrogen supply, but induce magnesium deficiency after prolonged application (Kibirige-Sebunya et al., 1996).

Ngambeki et al., 1992 reported that less than 20% Ugandan farmers used fertilizers in coffee production. Farmer low rate of fertilizer application range from unavailability, high price, slow adoption culture and being ill informed. Sometimes coffee farmers may use excessive urea, in times when there is an acute shortage of phosphatic and potash fertilizers. This unbalanced fertilization does not only deplete the soil of other nutrients but also results in the inefficiency of applied nitrogen and deficiency of other essential. Essential element deficiencies or absence lead to severe abnormalities in growth, development, or reproduction. With Uganda's annual population growth of 3.4% per year and the average of 6.7 children per woman, use of soil amendments to improve crop yields cannot be avoided. Regular soil fertility amendment in weathered and poor soils is one approach of increasing productivity and reducing land pressure. Since *Coffea* coffee thrives from shade to bright sunshine, sandy to humic soils, from very wet habitats (*C. congensis Froehner*) to arid ones (*C. rhamnifolia* (Chiov.) Bridson), the diverse agronomic traits expressed by genotypes in multi geographical habitats offers a great potential for phenotypic and genetic trait selection.

2.4 Uses of coffee by consumers and its environment implications

Other than health related factors, coffee has a multitude of uses. Traditionally, beans are chewed (masticated) and used for cultural and brotherhood relationships, offerings to gods and spirits in Uganda and other parts of Africa. The coffee plant is a good source of oxygen. Coffee is a good source of oxygen (Cannell, 1985; DaMatta et al., 2007). Ripe

cherries and hulls have been fermented to make wine or stewed to make a drink (Wrigley, 1988). Coffee pulp, reject seed, parchment, plant pruning or leaves is used as manure and mulch (Wrigley, 1988). Coffee wood is hard, dense, durable and used for furniture, fuel, construction among others. Both caffeine and chlorogenic acids (CGA) could be potential insect deterrents and chemisterilants. Caffeine too serves as repellant to predators and allelopathic compound (Uefuji et al., 2003).

The two main possible ways coffee improves health and reduces chronic illnesses is by use of antioxidants and caffeine. Cafestol and Kahweol antioxidant properties have shown some hyper cholesterol properties associated with human health (Leroy et al., 2006). Coffee antioxidants are known to affect the liver function, linked to heart disease and cancer protection. Chronic intake of unfiltered coffee raises diterpenes serum concentration. The total diterpene content in Arabica green beans is 1.3% wt/wt while that of Robusta is 0.2%. Polycyclic aromatic hydrocarbons from either green or roasted beans may be mutagenic and carcinogenic (Uefuji et al., 2003). Many of the secondary metabolites have real or purported commercial values such as insecticides; fungicides; pharmaceuticals, herbs, medicinals, dietary supplements and 'nutraceuticals,' flavorings and scents and other uses.

Transgenic caffeine-producing tobacco plants have enhanced disease resistance that helps minimize insecticide application (Ashihara et al., 2008). Though poisonous and bitter, caffeine is a mild addictive stimulant and diuretic substance that acts on the central nervous system, kidneys, heart and muscles. Coffee drinking increases brain performance, body metabolism, reduce risk of colorectal and liver cancers, asthma, type 2 diabetes, Alzheimer's and Parkinson's diseases as proposed in. Despite loss of key

flavour compounds, the 10% total consumed decaffeinated coffee offers similar health conditions aggravated by regular coffee because of high acidity (Dessalegn et al., 2008). Decaffeinated coffee has total diterpene content present in unfiltered coffee brews (Berthaud et al., 1988; Urgert et al., 1997).

2.5 Robusta coffee cropping in Uganda

Traditionally, Ugandan Robusta coffee farms are intercropped, commonly at random spacing with diverse crop species that are either vegetable, fruit (wild berries inclusive), legume, cereal, root crop or medicinal purpose. The common coffee shade trees include; Banana (*Musa* sp.), *Ficus sp*., Jackfruit (*Artocarpus heterophyllus*), pawpaw (*Carica papaya*), ovacado (*Persea americana*), *Albizia sp.,* Bark cloth fig (*Ficus natalensis*), Grevillea (*Grevillea robusta*), Mwafu (*Canarium schweinfurthii*), Guava (*Psidium guajava*), Muwafu (*African canarium* or incense tree), Nsambya (*Markhamia lutea*) Kirundu (*Antiaris toxicaria*) (Thomas, 1940). Variable spacing of Robusta coffee trees may have been adapted after thinning the forests. Some trees were planted at random before 3 meters by 3 meter spacing recommendation came to place. Farmers require ample space for coffee trees to co-exist with intercrop.

Shade requirements for coffee are determined by the ecological conditions of the field and locality. The different tree species conserve the ecosystem, promote survival capacity of the species, and provide food, building materials, fuel wood, or an alternative source of income and home for bees, birds and wild animals. Air temperature in a 40–50% shade is 7–10°F (4–6°C) cooler than in full sun (Elevitch et al., 2009). Shade trees ameliorate micro-climate for coffee bushes and moderate leaf temperatures and photosynthesis particularly during hot temperatures particularly during the two dry

seasons that normally last about 3 months. Tree shade prevents soil erosion, protects the coffee bushes from potential damage from hail storm, frost and wind and improves soil fertility by way of organic matter addition. The response of diseases and pests to shade is determined by the nature of shade and intensity. Through severe damage of leaves and flower and fruit shedding, strong winds, hail storm and frost depress crop yield, reduce leaf area and internode length of the orthotropic and plagiotropic branches (DaMatta et al., 2006). Lower air temperatures under shade may enable photosynthesis to continue even when open air temperatures are high. Meanwhile, hot winds increase crop evaporation and transpiration increasing rainfall requirements of trees. Shade leaves at 1-2^{o}C cooler than air temperature tend to develop darker leaves as a result of chlorophyll arrangement other than amount of chlorophyll per unit area (DaMatta et al., 2007).

With a provision for irrigation, tall trees canopy coverage of 30 % is adequate for flowering and backing effects while 40% sufficiently suffice without irrigation. Tree shade coverage of 45% decreased coffee tree productivity by about 20% (Vaast et al., 2006; DaMatta et al., 2006) and in so doing enable the plant bear the following year and farmer to harvest crop every year. Excessive production of fruits in one year followed by hardly any fruit production in next year (biannual bearing) is typical of open sunlight grown coffee. When accompanied by foliage reduction, physiological stresses such as overbearing may result to nutritional deficiencies, more parasite attacks, withered and dry branches and immature bean development. Excessive canopy drastically reduces yields to uneconomic levels. Coffee utilizes about 1% of the sunlight for photosynthesis. At leaf temperatures above 34ºC assimilation is practically reduced to almost zero (DaMatta et al., 2006), making photosynthetic rate of shaded coffee higher than open sun grown.

Optimum light intensity of 900 to 1300 E is required for maximum photosynthetic efficiency (DaMatta et al., 2006; 2007). Developing green leaves have 30 -60 stomata per mm^2 while mature sun leaves have 240 -260 per mm^2 (Browning, 1973a, b). For thick and strong coffee roots to develop, an extensive supply of nitrogen, calcium and magnesium is required (Wrigley, 1988). Clipping vertical roots before planting promotes horizontal root growth and access to water. Mechanization is not possible in tree shaded coffee fields.

2.6 *C. canephora* production constraints in Uganda

Coffee Wilt Disease (CWD) caused by *Fusarium xyalariodes* infects all *Coffea* species including the wild (Wrigley, 1988; (Adipala-Ekwamu et al., 2001) and therefore affects on the genetic base of *C. canephora*, including the development and sustainability of the coffee industry. Other than CWD, research selected varieties based on yield and acceptable quality succumb to pests such as coffee leaf rust (*Hemileia vastatrix* B. et B.), red blister (*Cercospora coffeicola* Berk et Cooke) coffee berry borer (*Hypothenemus hampei* Ferr) and of recent coffee twig borer (CTB), Xyalosandrus compactus. Another Robusta coffee production constraint includes low yielding varieties that are dominantly landraces (Wrigley, 1988). Farmer average yield of 600 kg/ha represents only 10-30% of what potentially can be achieved with improved crop husbandry (UCTF, 2008; 2009). Also the cup quality of cultivated Robusta coffee dominated by landraces has not been determined or profiled coupled with identification of superior genotypes for promotion or quality improvement. Agronomic recommendations should involve improvement of degrading coffee soils, shade and water supply options to ameliorate drought. Robusta coffee physiology needs to be understood under varying environmental conditions. To ease

utilization and increase chances of attaining success in any agronomic improvement, we need a variable gene pool with known traits which at present is limited. Worse still, population pressures and economic hardships are threatening genetic diversity reservoirs in the wild and in landraces in farmer fields.

2.7 Genetic diversity of coffee

One important and cheaper source of Robusta coffee "genetic pool" reservoirs for crop improvement are natural populations in the wild and farmer landraces where continued generation of variability is derived through selection, mutation, drift, gene flow and mating systems (Brown, 1995. The largest available Robusta germplasm collection at Kawanda consists of selected out crossed progeny of original parent stock which unlikely represent immense genetic diversity existing at farmer fields and the wild (Thomas, 1940; Wrigley, 1988). More Robusta coffee accessions from forests and landraces from farmer fields need to be assembled to enrich Kawanda germplasm collection. Research may introduce coffee germplasm with established genetic traits to enrich local collections. Apart from intra crossing within *C. canephora* (Robusta), hybridizing diploid *Coffea* species is possible because gametophytic self in-compatibility would enable exchange of genetic material to generate variability. Making crosses with Hybrid de Timor, a known natural mutant between tetraploid Arabica and diploid Robusta (Leroy et al., 2006) offer opportunity of increasing quality among other desirable traits. In Uganda, tetraploids from the best Robusta selections were developed in 1972 by use of colchicine chemical (Wrigley, 1988). Arabusta hybrids were back crossed to Arabica coffee to restore cup quality while maintaining resistance to coffee berry disease and coffee leaf rust.

Apparent valuable products of coffee genetic resources in Uganda is the agronomically selected line No.9 from Toro germplasm collections whose progeny constituted part of the seed distributed to farmers in the 1920s (Thomas, 1947). The self pollinated seedling progeny of No. 9 (9/6) with follow up selections contributed to the development of the eight commercial Robusta coffee varieties ($1^{s}/2$, $1^{s}/3$, $1^{s}/6$, 223/32, 236/26, $257^{s}/53$ and $258^{s}/24(0)$, 259s/56 (Wrigley, 1988; Kibirige-Sebunya et al., 1996) that later succumbed to coffee wilt disease (CWD). Clones 223/32 and 259s/56 were withdrawn in the 1990s because of high coffee leaf rust infestation. Rigorous selection of coffee wilt disease resistant varieties since 1993 in Uganda also utilized wild, landraces and germplasm collections to curb the diseases and minimise chemical use. Economic and sustainable coffee production in Uganda has been made possible in the midst of devastating disease outbreaks because resistant mother trees were selected from heterogeneous forest (wild) and garden (landrace) coffee populations. This suggests that it is possible to find desirable traits from wild and landraces for conservation and regular crop improvement (Charrier et al., 1985).

C. canephora like *C. arabica* and other wild coffee species and semi wild forms are diminishing as a result of widespread dependence and increasing impact of growing populations. Deforestation has been accompanied by loss of valuable genetic resources and biodiversity. Besides, income is lost with indiscriminate cutting of forests because in Uganda forest coffee is still harvested and sold (Thomas, 1940; UCDA, 1991-1993). Uganda did not benefit from international coffee germplasm collection and conservation missions of FAO (Food and Agricultural Organization) in 1964-1965 and IBPGR (International Board for Plant Genetic Resources) in 1983-1989. French coffee

conservation missions included ORSTOM, (presently IRD – Institut de rescherche pour le d_eveloppement) during 1966-1987, CIRAD (Centre de cooperation internationale en recherche agronomic pour developpement) in 1960-1987), Museum of Natural History, Paris, France from 1960-1974 (Anthony et al., 2007). In Cote D' Ivoire, 8000 reference living collections of *C. canephora* wild forms, local varieties and populations from farmers and selected materials have been conserved (Charrier et al., 1985; Anthony et al., 2007). Another 25 diploid coffee species have conserved *in situ* in Madagascar.

Like other crop species, understanding the genetic structure and trait composition of Robusta landraces is significant as it reveals the economic potential and provides information for strategic planning, utilization, ecosystem conservation and survival capacity of the species. Limited knowledge of existing genetic base makes Robusta coffee improvement unnecessarily long, expensive and one that has limited success.

2.8 Methods used to evaluate genetic diversity

2.8.1 Genetic diversity approaches

Genetic diversity studies on populations, breeding lines, germplasm collections are aimed at establishing existing variability, identify diverse parental lines to generate segregating progenies and identify desirable genes for introgression. One or more methods have been used to analyze numerical data sets such as DNA, morphology, agronomic performance and biochemical compounds (Mohammadi et al., 2003). Except for DNA, the rest of data sets are usually influenced by inherent and environmental factors. Sampling strategy, strengths and weaknesses of data sets, choice of genetic distance and study objective are some of the important technical considerations. One has to reflect on experiment objective, level of resolution, infrastructure, available time and resources.

Several methods have been utilized to investigate the genetic diversity of coffee or any other crop. Attribute mean, range measures, standard deviation (S^2-variance estimated for a sample), variance (O^2-population), degrees of freedom and coefficient of variation (% ratio of standard deviation to mean that compares different sample means of a population) provide the extent of attribute variability (Fowler et al., 2001). Analyses of variance (ANOVA) compares several sample means to determine significant differences. Genetic diversity evaluations also consider distinct allele numbers and their allelic frequencies distributed at separate loci (equated as Polymorphism Index Content) and Nei's unbiased estimate of gene diversity (H) (Nei, 1987). The advantage of using Polymorphism Information Content (PIC) in measuring genetic diversity is because it is independent of sample sizes (Muchugi et al., 2008). Chi squared tests (X^2) using frequency based statistics can be used for detecting differences between populations or population subdivision when mutation rate and allelic diversity is low. The extent of between and within population differentiation can be established using F statistics of Wright, 1978. Wright's approach of estimating F coefficients considers the following: (a) correlation of genes within individuals over all populations (F_{IT}), (b) correlation of genes of different individuals in the same population (F_{ST}) and (c) correlation of genes within individuals within populations (F_{IS}). F_{ST}, F_{IT} and F_{IS} values are therefore interrelated. Because biological entities do not stand on their own but depend on each other, there is need to determine genotypic relationships and effects that could be environmentally influenced.

2.8.2 Genetic distance measures

Genetic distance (GD), sometimes called a metric, is the gene difference between populations or species measurable in some numerical quantity (Nei, 1987). Euclidean or straight-line measures of genetic distance such as GD_{SM} (genetic distance derived from simple matching coefficients) or GD_{MR} (modified Rogers' genetic distance) are some of the statistic used for estimating genetic distance between individuals (genotypes or populations) (Mohammadi et al., 2003; Perrier et al., 2003). GD_{MR} is widely preferred because of its powerful genetical and statistical properties. GD_{SM} has Euclidean metric properties that allow its use in hierarchical clustering strategies creating minimum variance within a group as proposed by Ward (Mohammadi et al., 2003). The analysis of molecular variance, AMOVA (Muchugi et al., 2008), makes use of F related statistics to structure genetic variation in form of variance components among and within groups. GD_{SM} is limited in that it gives equal weight to both 0-0 and 1-1 matches in case of binary data. While 1-1 match indicates more similarity, the reasons for 0-0 match are many; lack of amplification, absence of bands, lack of identity by descent or by state. Other genetic distance or genetic similarity (GS) measures commonly used to estimate binary DNA data include Nei's coefficient (GDNL) (Nei et al., 1979) and Jaccard coefficient GDJ) (Jaccard, 1908). Gower, 1971 also described a general coefficient for measuring genetic distance between individuals on the basis of qualitative, quantitative and dichotomous data as; 0 wherever there is a match between two qualitative characters and 1 when there is a mismatch. The distance between two quantitative characters was calculated as the difference in the trait values divided by the overall trait range and converting the distance for quantitative characters to a scale of 0 to 1. Multiple Correspondence Analysis (MCA) have also been used to calculate X^2 distances on a

binary table arrangement for qualitative variables with modalities like more, equal and less (Perrier et al., 2003).

Appropriate choice of a genetic distance measure depends on the type of variable and the scale of measurement. For co-dominant markers (such as RFLPs and SSRs), the expected genetic distance of Nei et al., 1979 coefficient relates pairs of lines as linear function of their co-ancestry coefficient (Perrier et al., 2003). For dominant markers, Jaccard's genetic distance can be applied but not GDNL (Link et al., 1995). In case of codominant markers among inbred lines, both GDJ and GDNL may lead to identical ranking of GD estimates. Both GDJ and GDNL suffer from unknown statistical distributions because the denominator used to calculate sampling variance and confidence interval is a random variable.

To overcome the problem of unknown statistical distribution in measuring parameter genetic variance, the bootstrap or Jackknife techniques are used to empirically estimate sampling variance using a few units that provide little information for distribution (Efron et al., 1979). Bootstrap method can estimate errors and confidence intervals for the mean, median, proportion, odds ratio, correlation coefficient or regression coefficient. Bootstrap method can construct a hypothesis or make a parametric estimate. Meanwhile, the Jackknife method is shown to be a linear approximation method of a bootstrap. The limited number of re-sampling units makes the Jackknife method provide limited statistic distribution frequency. The re-sampling techniques estimate a wide and best statistic distribution frequency from which they were drawn based on independent variables, standard error, confidence limits and other statistical methods of accuracy that are not available or difficult to calculate.

The statistical accuracy in bootstrap analysis is generated from repeatedly sampled equal size of original sample that provides statistic of interest for each bootstrap sample produced (Efron et al., 1979; Mohammadi et al., 2003). Bootstrap sampling is also used to determine reliability of tree topologies or genetic distance values in a dendrogram. Genetic distances of 70% and above are likely to be 95% correct. In case of need for more than one measure of genetic distance to analyze a given data set or different data sets, Mantel test can be used to ascertain the correspondence between matrices derived from different distance measures (Mohammadi et al., 2003). Bray-Curtis distance measure can be applied to estimate pooled similarity percentage variance contribution of each variable to sub group formation and mean group abundance in K means analysis derived groups (Perrier et al., 2003).

2.8.3 Cluster analysis

Factorial or multivariate analyses are useful in species or population diversity studies because they unveil the most influential characteristics in genotypes (Perrier et al., 2003). Multivariate analysis methods have gained significance in classifying many accessions by ordering genetic variability or analyzing genetic relationships among breeding materials based on the characteristics they possess (Mohammadi et al., 2003). Multivariate statistical algorithms simultaneously analyze multiple measurements of each individual investigated (whether morphological, biochemical or molecular marker data). Individuals with similar descriptions are mathematically gathered together providing clusters with high internal (within cluster) homogeneity.

Clustering algorithms such as the principal component analysis (PCA), principal coordinate analysis (PCoA), and multidimensional scaling (MDS) are at present most

commonly employed and appear particularly useful (Perrier et al., 2003). These ordination methods produce a geometric representation of individuals with each genotype differing from the mean by its own mean characteristics. Ordination methods facilitate the detection of individuals or populations that show some intermediacy between two groups. The sum of squares of each unit from the mean point is what the factorial analysis quantifies as inertia of dispersal.

Two major clustering methods are commonly utilized, the model and the distance based methods. The model based method of clustering assumes observations from each cluster are random draws from some parametric model, and inferences/deductions from parameters corresponding to each cluster and cluster members are performed jointly using standard statistical methods such as maximum-likelihood or Bayesian methods (Mohammadi et al., 2003).

The distance based method of aggregating genotypes uses a pair wise distance matrix as an analysis input for a specific clustering algorithm (Mohammadi et al., 2003; Perrier et al., 2003) giving a graphical representation such as a tree or dendrogram. Hierarchical clustering methods such as UPGMA (Unweighted Paired Group Method using Arithmetic averages) and the Ward's minimum variance method (Mohammadi et al., 2003) are commonly used in genetic diversity analysis in crops to merge a series of smaller groups of individuals starting with the most similar. In UPGMA clustering, a Factorial Analysis of Dissimilarity Table (FADT) uses a dissimilarity matrix data of genetic distances to graphically represent a heterotic tree groups or dendrogram (Saitou et al., 1987). UPGMA provides consistent heterotic and pedigree information on biological materials and clusters that relate to genotypes of different data sets. Ward's method

avoids the chaining effects often observed with UPGMA. Both Ward and UPGMA are suitable for similar and different group size analyze (Mohammadi et al., 2003).

The nonhierarchical procedure, frequently referred to as K-means clustering, are based on sequential threshold, parallel threshold, or optimizing approaches for assigning individuals to specific clusters, after specifying the most suitable number of clusters (Mohammadi et al., 2003; Perrier et al., 2003). Lack of prior information on cluster number constrains the use of this method. Descriptive statistics are useful methods for characterizing specific diversity groups using the mean, median, variance and inter quartile range in form of box plots.

Though many clustering methods exist such as UPGMA, UPGMC (Unweighted Paired Group Method using Centroids), Single Linkage, Complete Linkage and Median, a single clustering method may not reveal genetic associations effectively and each method has relative strengths and constraints. For instance UPGMA and UPGMC seem comparable with a relatively high level of accuracy for pedigrees while Single Linkage and Median clustering methods are associated with the chaining effect and poor resolution of individual groups that complicate the interpretation of results (Mohammadi et al., 2003). To compare the efficiency of different clustering algorithms, cophenetic correlation coefficient can be used to measure the agreement between the dissimilarity indicated by a dendrogram and the distance similarity matrix as input of cluster analysis. A method with the highest cophenetic correlation coefficient is regarded as the best (Mohammadi et al., 2003).

Meanwhile a factorial step discriminant analyses based on the principal components calculates Mahalanobis distance (D^2) between group centroids (vectors of means) to identify the best clustering algorithm and to verify whether the derived groups were significantly different or qualify to be different populations. The best clustering method produces the largest distance D^2 among groups or clusters and is appropriate for quantitative data. Fisher inter-group distances and significance also measure genetic diversity. The disadvantage of clustering methods is that the algorithms do not define what constitutes an optimal tree or dendrogram and systemic errors incurred during cluster analysis reconstructions. Use of neighbor joining removes the assumption that the data are ultrametric (Perrier et al., 2003). Euclidean distances and particular distances are examples of ultrametric distances. The neighbor joining method has commonly been used for phylogenetic studies but not for intraspecific differentiation in crop plants (Mohammadi et al., 2003).

Combined data analysis can therefore reveal information common to all data sets or specific to a given table. Before combining different data sets it is most important to consider the similarity or connection among the results derived from individual data sets to ascertain whether a better estimate of genetic diversity will be obtained with combined data sets. Qualitative data can be assessed using Multiple Correspondence Analysis (MCA) and the quantitative tables by PCA. Combining data from qualitative and quantitative measures possibly biases distances estimated on the basis of quantitative characters and increases the level of correlations among qualitative characters. The most highly structured tables may adversely render the input of the more loosely structured tables. It is advisable to assign weights to qualitative characters for clustering purposes

to reveal their contributions to genetic diversity in individuals or populations (Mohammadi et al., 2003). With use of a Modified Location Model (MLM), all the categorical variables can be combined into one multinomial variable W, which can be then used with the available continuous attributes (Mohammadi et al., 2003).

To extract maximum information from the molecular marker data, ordinations such as PCA or principle coordinate analysis (PCoA) can be used in combination with cluster analysis, particularly when the first two or three PCs or PCoA explain over 25% of the original variation (Mohammadi et al., 2003; Muchugi et al., 2008). With relatively few characters and no missing data, the output of PCA and PCoA are similar. PCoA is a better clustering option with missing data because it replaces missing values with independently computed coefficient of two individuals while the PCA uses mean values. The PCA is very useful for solving pattern recognition problems arising from diversity studies, chromatographic and spectroscopic data (Perrier et al., 2003; Muchugi et al., 2008).

Varimax rotation imbedded in statistical programs such as XLSTAT version 2011.2.05 (Addinsoft SARL Company, Paris, France) was used to improve the principal component analysis plot reliability (Perrier et al., 2003). Multidimensional scaling (MDS) or perceptual mapping of genotypes in a few dimensions is applicable when use of hierarchical algorithms gets limited with nonhierarchical and reticular patterns of diversity. In MDS, linearity is not assumed (Mohammadi et al., 2003). Like allele frequencies, phenotypic parameters are used for pair wise comparison of accessions to derive a matrix for multi dimensional scatter. Unlike data with uniform parameter scale measure such as DNA, bean biochemical compounds, organoleptic cup sensory rating

and nutrient elements and parameters with different scale such as morphology, bean physical characters) utilize a correlation matrix to standardize original data.

2.9. Why XRF and NIRS are future techniques

Typically, X-rays are used to reveal the chemical composition whereas near ultraviolet to near infra red wavelengths distinguish the configurations of various isomers in detail (Brouwer, 2003). X-rays and Near Infra Red Spectroscopy (NIRS) technique complement each other. Both X-Ray Florecense (XRF) and NIRS spectroscopy are non destructive, sensitive and rapid quantitative and qualitative techniques of analyzing the fluorescent x-ray or light to investigate sample element composition or identify compound physical structure which would ordinarily be invisible to the eye (Brouwer, 2003). Drug metabolism studies have also applied spectroscopy techniques (Kostiainen et al., 2003). Additionally, NIRS authenticates products according to fingerprint (Downey et al., 1994; Davrieux et al., 2005; Bertrand et al., 2005b; Huck et al., 2005).

X rays and NIRS have been used as rapid characterization tools with diverse applications in medical and pharmaceutical imaging. XRF benefits also outweigh relative requirement of adequate sample volume for destructive grinding and pressing to make a pellet, or fuse with inert material to form glass bead and time consumption are other draw backs (FitzGerald et al., 2008). At low concentrations, NIRS technique cannot fully quantify substances. In such situations, precise and verification purposes may use the more expensive, complex and time consuming analyses, for example by High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) be warranted.

2.10 Factors that influence coffee quality

2.10.1 Definition of coffee quality

Coffee quality is a complex trait to measure because numerous factors such as plant genetics, technical handling procedures, ecological conditions and agricultural practices influence quality characteristics along the value chain. International Organization for Standardization (ISO) describes quality as "the ability of a set of inherent characteristics of a product, system or process to fulfill requirement of customers and other interested parties" (ISO, 2000). Depending on the concerned party along the chain (Leroy et al., 2006), coffee quality could imply variety, price of coffee culture, consistent supply, tonnage or bean physical characters and biochemical compounds. Effects on health and sociological aspects are equally important. Cup quality is what determines commercial coffee grade, but not necessarily bean size. ISO (2004a) has defined a standard for green coffee quality (ISO 9116 standard) and methods of measuring qualities to include defects, moisture content, bean size, chemical compounds and cup tasting.

2.10.2 Bean physical characters

Coffee green bean (seed inside hull) physical characters such as weight, volume, size, shape, colour, solubility, moisture content and texture are usually evaluated to provide indicative information on cup quality. Different markets prefer different physical bean characters provided they have acceptable visual aspect, are homogeneous and their production is regular and reliable. Inherent traits and growth conditions determine bean physical characters (Leroy et al., 2006). Different environments and farm management practices have significant contribution to bean physical characters, biochemical composition and to human health (Lu et al., 1997; Davrieux et al., 2003). Robusta coffee

beans from high altitude may be greenish in colour, small, denser, harder and more appreciated due to higher acidity, good aroma and flavour than that from lower altitudes.

2.10.3 Green bean biochemical compounds

Biochemical compounds that determine coffee cup quality arise from precursors in green beans during development (Davrieux et al., 2003) and are genetically and environmentally influenced (Montagnon et al., 1998; Leroy et al., 2006). These biochemical compounds help discriminate the different coffee types making them key factors for organoleptic cup quality. A large variation in bean biochemical compounds has been observed in over one hundred genus *Coffea* species (Clifford, 1985) with *C. canephora* being less aromatic and richer in caffeine than Arabica coffee. High chlorogenic acid and caffeine content make the coffee liquor bitter, while high sucrose and trigonelline contents produce better aroma and fat content help fix flavour compounds formed during roasting (Davrieux et al., 2003; Charrier et al., 1985). High chlorogenic acid is associated with high caffeine content (Campa et al., 2005 a, b). Caffeine, a byproduct of chlorogenic acid and purine bases is probably produced in response to stress and attack as a defense mechanism (Ashihara et al., 2008; Uefuji et al., 2003). Both caffeine and chlorogenic acid contribute to the astringent and bitter coffee brew. Trigonelline is 100% water soluble and probably the most significant constituent contributing to excessive bitterness in coffee (Berthaud et al., 1988; Ky et al., 2001b). *Coffea arabica* has more trigonelline content than *C. canephora* (Charrier et al., 1985). Different levels of narrow sense heritability have been reported for Robusta coffee green bean biochemical compounds as follows; sucrose content (0.11), fat content (0.74), trigonelline (0.39), caffeine (0.80), chlorogenic acid (0.36) and bean weight (0.73) (Montagnon et al., 1998).

Robusta coffee biochemical groups relate greatly to molecular groups (Leroy et al., 1993). Attributing any of the over 800 aromatic compounds present in roasted coffee to any flavour is not practically viable (Wintgens, 2004). As a result, indirect predictors such as chemical compounds (sugars, lipids, proteins, chlorogenic acids, and methylxanthines) through the traditional wet chemistry method and indirect methods like Near Infrared spectra (Bertrand et al., 2005b) have been engaged to access coffee quality.

According to Silva et al., (2005) elevation but not soil water availability influenced bean biochemical composition. Temperature influenced fatty acid composition during coffee bean development (Villarreal et al., 2009; Joet et al., 2010). High leaf to fruit ratio reported in higher elevation arises from a longer leaf life span (Vaast et al., 2006). Air temperature (Joet et al., 2010) and elevation (Bertrand et al., 2006) increased chlorogenic acid during seed development. Altitude increased fat concentration in Arabica traditional varieties (Bertrand et al., 2006). Higher sucrose, chlorogenic acid and trigonelline concentrations have been observed in sun-grown than shade-grown beans possibly due to incomplete bean maturation and account for increased bitterness and astringency in the coffee beverage (Muschler, 2001). Glucose levels were positively affected by altitude, while sorbitol content after wet processing depended directly on the glucose content (Joet et al., 2010). Temperature influences phenologic cycle of coffee, particularly berry development and ripening (Guyot et al., 1996), athough some reports revealed that early picked ripe cherry of C. *arabica* in Costa Rica gave the best quality (Muschler, 2001) Delayed berry flesh ripening encountered in low temperatures at higher elevations, allowed for longer and better bean filling that improved bean size, chemical contents and cup quality than yellow or green cherries of *C. canephora* picked earlier (Guyot et al.,

1988; Vaast et al., 2006). Over-bearing potentially reduces bean size as a result of carbohydrate competition among berries during bean filling (Cannell, 1985; Bertrand et al., 2004; Vaast et al., 2006).

2.10.4. Organoleptic cup quality

Though coffee visual appearance is an important indicator of quality, it is not reliable. Final coffee quality depends on cup quality as assessed by human sensory organs and consumers who have a specific taste according to their nationality (Leroy et al., 2006). Usually attributes such as fragrance, aroma, flavour, bitter, sweet, salt, acid, mouth feel, aftertaste, balance are normally evaluated. Smell of roasted dry or ground beans provide fragrance while aroma is smelt upon brewing with boiled water. Aroma helps evaluate flavor and coffee liquor brightness (Clarke, 1986). Microclimate and soil contribute to coffee flavor profile (Wintgens, 2004) which is an attribute of appreciating the coffee brew. More dissolved coffee solids make the coffee brew bitter. High levels of potassium in Robusta coffee are associated with brackish (high saltiness and un pleasant aroma) aftertaste while low potassium levels in coffee brew are savory (low saltiness and pleasant aroma). When both potassium and caffeine are at lower levels, the coffee brew taste is coarse and harsh (Clarke, 1985). At medium roast, coffee has less soluble solids, a higher acid content, and a potent aroma as compared to the dark roast (Wintgens, 2004). With bitter sweet aspect ratio, low bitterness with high sweet combination is rated high.

Perceived acidity in coffee is a result of acids such as aliphatic, chlorogenic, alicyclic carboxylic and phenolic acids that are developed best at medium dark roast stage. Citric acid, malic acid, and acetic acid could be the most important factors in cup acidity because they exist in high proportions and have low phosphous and potassium.

Phosphoric acid has been suggested as a major contributor to acidity though it may not directly correlate to perceived acidity (Clarke, 1987). Soft, pleasing and delicate taste is a characteristic of cup acidity and sweet coffee obtained from bean acids, high sugars levels and chlorogenic acids (Maier, 1987). Mouth-feel or liquor body is determined by micro fine fiber and fat content while texture is derived from oils extracted from ground coffee and suspended in the brew. Brew colloids form when oils coagulate around fibers suspended in the brew and are helpful in assessing coffee weight and texture (slipperiness). Viscosity is the brew is caused by proteins and fibers which are normally heavier in medium and dark coffee roast than lighter roast. Taste is the feeling in the mouth after sipping the beverage while aftertaste is a changing sensation experienced at the back of the throat after swallowing. Normally the tongue perceives sweet, salt, bitter, and sour taste attributes. A complementary synergistic combination of flavor, aftertaste, mouth feel and bitter/sweet aspect ratio is rates as a balanced cup.

Roast bean fat correlates with aroma, body, acidity, flavor, aromatic intensity and quality, overall judgment and preference (Charrier et al., 1985; Davrieux et al., 2003; Barbosa et al., 2005, Larcher, 2005; Decazy et al., 2003; Tessema et al., 2011). When vigorous and productive trees yield in excess beyond what the tree can adequately nourish under favourable conditions, beans with reduced acidity are produced. Caffeine content has been found to be negatively and significantly correlated with cup quality attributes (Dessalegn et al., 2008). Moschetto et al., 1996 reported linear correlation coefficients between preference and acidity and aroma in Robusta coffee hybrids and commercial clones. Based on studies by Leroy et al., 2006, cup acidity and aroma could be used as

selection criteria for Robusta organoleptic quality since they are easier to define and select.

High quality coffee markets demand distinctive cup attributes that are homogeneous, regular and reliable. Organoleptic cup attributes ought to be stable, especially for the roaster and the consumer (Leroy et al., 2006). Other than enotype and environment (Barbosa et al., 2012) cup quality is further accelerated by how cherries and beans were handled between picking, shipping and roasting. Varying cup differences that result from genotypic differences contribute greatly to market, for example buyers in Central America prefer traditional varieties (Bourbon, Caturra, Catuai, Pacamara) to newer varieties derived from the 'Hybrid of Timor', an inter-specific hybrid between *C. arabica* × *C. canephora*. In Uganda, where *C. canephora* has evolved over years and traditionally cultivated as a culture, farmers and buyers are skeptical of Arabusta hybrids selected based on quality and other desirable agronomic traits even at the peak of coffee wilt disease. Genotypes portray different cup qualities under different environments. For instance Blue Mountain provides superior liquor quality when grown in Latin America than in East Africa (Leroy et al., 2006). Generally African coffee has high acidity, low body, a distinct bright colour of citrus, sweet fruits, floral and a dry wine taste for example in Uganda, Ethiopia, Tanzania, Kenya, Zimbabwe and Zambia. Coffee from Asian countries such as India, Java, Sumatra, Sulawesi and Papua New Guinea has low acidity, high body and smoothness, earthy and spice flavour characteristics. Countries from Latin America such as Brazil, Columbia, Costa Rica, Guatemala, Nicaragua, Mexico, El Salvador, Peru, Panama and Honduras produce coffee with medium acidity

and body, intense aroma with a full spectrum of tastes that range from fruit to earth and nut, vanilla and chocolate. Other than genotype and crop husbandry effects, organoleptic cup differences in various continents is attributed to environmental conditions such as soils, temperature, altitude (Wintgens, 2004).

2.10.5 Effects of coffee farm management on coffee quality

The genotypes the farmer desires to have in their field and how they are managed influence tree growth and production. Since landraces dominate most cultivated coffee in Uganda, tree existence depends on farmer choice which is based on yield and other desirable agronomic biotic an abiotic traits. Tree canopy management like pruning helps check biennial bearing towards systematic but moderate yields. Non pruned and desuckered coffee reduce yield, are more humid with pronounced effects of fungal and pest infestations that make the beans deteriorate into immature or damaged fruits (Wrigley, 1988.) Flies such as *Ceratitis coffeae* introduce bacteria into the bean that produces a potato taste with 2-methoxy-3-isopropyl-pyrazine molecule. Damage by diseases such as red blister and coffee berry disease, dry out the fruits or make it rot. Sanitary, proper and timely applications of pesticides minimize damage. Excessive use of potassium-rich *Pennisetum purpurem* as mulch introduces excess potassium fertilizer that can lead to harsh beverages. Too much nitrogen fertilizer increases the caffeine content of beans, leading to increased bitter beverage. Chemical treatment of infected beans if not properly done gets expressed in the coffee brew (Clifford, 1985).

2.10.6 Coffee bean processing

Harvest and post harvest processing also influence bean quality (Clarke, 1985). For instance, glucose content was positively affected by altitude, while sorbitol content after

wet processing depended directly on the glucose content in fresh seeds (Joet et al., 2010). Selective picking of physiologically ripe coffee avoids a bitter and a stringent cup produced by physically premature beans. In Uganda, Robusta coffee is commonly dry processed and called ("Kiboko"), while Arabica is wet processed. Dry processed Robusta coffee brew has body, sweet, smooth and complex. Increased body of dry processed coffees masks cup acidity. Dry processing is generally avoided for quality samples as it enhances bitterness in the liquor. In cases where the Robusta coffee is wet processed, the coffee brew derived is cleaner, brighter and fruitier. Some other processing methods that Ugandans could adopt to improve their coffee quality or diversify their coffee products include pulped natural or re-passed methods. With pulped natural method, pulped coffee is not fermented to remove the silver skin. Pulped natural brew is sweeter than wet-processed coffees, has body of dry-processed coffee and retains some acidity characteristics of wet processed coffee. Low humidity is required for the mucilage to dry rapidly before fermenting. The re-passed or raisins method uses coffee that dries in the trees before collection. Re-passed coffee has high flavor profile and much sweeter than traditional pulped coffees. They can be washed or used as pulped naturals.

In all the drying and storage process, caution should be taken to avoid water re-absorption or exposure to humid environments. Removal of defect beans such as cracked, withered, moulded beans improves coffee quality. Coffee beans have a porous and spongy texture which makes them pick up strong odours and absorb water easily. Moisture content in fresh cherry is estimated at 50%, green bean 12.5 % depending on humidity. Moisture content of up to 12.5% is recommended for storage (Clifford et al.,

1985; Clarke et al., 1985) to minimize contamination. Porous and spongy texture of coffee beans (Priolli et al., 2008; Wang, 2012) encourages absorption of odours and water that encourage fungi growth and rapid deterioration (Clifford et al., 1985; Clarke et al., 1985). When acidity gets lost with long time storage, the coffee is described as aged. High moisture content makes the coffee age fast with excessive loss of weight during roasting. The pulping or hulling machine should properly be designed and adjusted to minimize chipping or crushing of beans. With roast temperatures of above 200°C, the coffee bean undergoes dehydration and chemical reactions generate gas and make beans brown. About 16 % of water is lost with a 50-60% volume increase in beans (Clifford et al., 1985; Clarke et al., 1985). Roasting develops and attains desired chemical, physical, structural and sensorial changes (Maier, 1987; Clarke et al., 1988). Roast bean color change and weight loss frequently estimate the roast degree and final roasting temperature. Smell and physicochemical bean roast characteristics are used to target unique aroma, flavour profiles and structural product properties of specific brands. Long roast time at low temperature is known to produce sour, grassy, woody and under developed flavour properties. A short roast time at high temperature generates high quality coffee with more aroma, moisture content, pH value, titratable acidity and porous bean cell structure. Additionally, a short roast time at high temperatures produces more flavor, aromatic acids and caffeine (Wang, 2012). Roasting can be done using hot gas in conventional horizontal or vertical drums for 3-12 min at 230-250^{o}C. In a wet bed, beans are roasted using a high velocity hot air of 230-360^{o}C usually from the bottom of the roaster that suspends beans in turbulent air.

There are four major reactions that determine to a great extent the aroma of roasted beans. Firstly the Maillard reaction occurs between nitrogen containing substances such as amino acids, proteins, trigonelline and serotonine with carbohydrates like sugars. Degradation of individual amino acids, particularly sulphur amino acids, hydroxy-amino acids and praline is the second reaction. Thirdly, sucrose degrades to compounds like aliphatic acids and caramel-like substances that contribute to flavor either as volatile aroma compounds, or non-volatile taste compounds (Ginz et al., 2000). Fourthly phenolic acids particularly the quinic acid moiety are degraded. Other reactions involve degradation of lipids and interactions between degraded products (De Maria et al., 1996). The resultant pyrroles created eventually change the chemical composition of the bean giving rise to coffee aroma as well as release carbon dioxide. At medium dark roast colour, roasted beans develop an oily sheen from caramelizing sugars.

Among coffee aromatics, furans are the most predominant suggested compounds that likely impact on coffee aroma due to their typical caramel-like odors that result from sugar pyrolysis (Clarke, 1985; Wintgens, 2004). At roast, furans also produce key aroma notes when reacting with sulphur containing compounds (Maier, 1987). Pyrazines aromatic compounds contribute to the roasted, walnut, cereal, cracker, or toast-like flavors in coffee.

CHAPTER THREE: GENETIC DIVERSITY OF *Coffea canephora* L AS REVEALED BY SIMPLE SEQUENCE REPEAT MOLECULAR MARKERS.

3.1 Introduction

Understanding genetic variability is a key pre-requisite to targeted improvement of the lucrative global coffee industry. A variable genetic base provides a pool of traits for crop improvement. Breeders continuously source and make selections of alleles against biotic and abiotic stresses while improving crop quality. Knowledge of diversity structure is important to best exploit and conserve the genetic resources (Leroy et al., 1993). Globally, coffee genetic resources are conserved through either in-situ or ex-situ.

In Uganda, *C. canephora* has been observed agro ecologically and genotypically to be variable (Maitland, 1926; Thomas, 1940; (Charrier et al., 1985). Using Ugandan wild Robusta coffee, Musoli et al., 2009 identified another subgroup of the Congolese main genetic diversity group constituting cultivated (nganda and erecta), feral from Kalangala, Itwara and Kibale forests that could form important and natural cheaper sources of genetic pool reservoirs for crop improvement. Robusta coffee is reported to be endowed with several economically useful traits that range from tolerance to diverse pest invasions to high crop production and productivity among others (Prakash et al., 2005). With the looming scenario of genetic resource erosion, existing heterogeneous genetic resources will be wiped out before they are characterized and protected.

Molecular markers have been used to evaluate the genetic diversity of many field crops (Berthaud, 1986; Prakash et al., 2005). A molecular marker by definition is a small DNA fragment with a precise position within the genome, showing genetic differences between

individuals of the same species (alleles). In recent years, different marker systems have been developed and applied to a range of crop species including coffee to speed up research investigations without the influence of non-genetic variations making them more preferred than traditional morphological descriptors. The information generated from evaluation of coffee DNA has increased knowledge on coffee genetics and understanding of the structure and behaviour of coffee genome.

To investigate coffee genetic diversity in detail and select cultivars with desirable traits, selectively neutral genetic markers like Restriction Fragment Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphisms (AFLP) and Simple Sequence Repeats (SSRs) among others have been used. Genetic molecular markers evaluate the genotype as close to the DNA as possible, allowing for the level of resolution that extends to intra-species differentiation. Genetic markers provide good insights to questions related to phylogenetic relationships, key evolutionary processes such as gene flow, mating systems and population size than ecologically important traits without non-genetic variance that often makes other trait analyses uncertain (Lu et al., 1997).

3.1.1 Simple Sequence Repeats (SSRs) in Coffee

SSR selectively neutral genetic marker, comprise repeated pattern of mono, double, tri and tetra nucleotides (small DNA fragments) that allow us to amplify the precise position within the genome that reveals a series of several alleles (polymorphism) for each locus studied (Table 4.3). This precision is guaranteed by short primers (20–25 nucleotides) that flank the SSR region (Grivet et al., 2003). Microsatellites DNA polymorphisms have been used to estimate genetic variability characterize and identify cultivars with specific

traits. DNA evaluation can also be used to identify hybrids and foreign DNA segments as well as build species genetic linkage maps (Lu et al., 1997; Tsegaye, 2002). SSRs are easy to type, co-dominant, repeatable, polymorphic, follow Mendelian inheritance, can handle more samples per unit time and analyses can easily be combined with other techniques. When SSRs are linked to the gene loci of interest they can be used in marker assisted selection and are amendable to recent advances in genomic and bio informatics. One of the handicaps with polymorphic SSRs is that they require more effort than any other Polymerase Chain Reaction (PCR) based DNA survey. The characteristic small size (20–25 nucleotides) of SSRs allows amplification of precise position within the genome revealing several alleles for each locus studied (Grivet et al., 2003). Several studies have reported the use of SSR DNA polymorphisms for quantifying genetic variability, characterizing and determining the structure (Prakash et al., 2005).

SSR DNA polymorphisms have been used to quantify genetic variability, (Tsegaye, 2002). SSRs have revealed more alleles per polymorphic loci and higher differentiation in *C. canephora* (Aga et al., 2003; Prakash et al., 2005). Polymorphic SSRs with respective primer sequences have been developed to reveal genetic polymorhism in *Coffea* and Psilanthus species (Combes et al., 2000).

3.2 Materials and Methods

3.2.1 Sample collection

A total of 231 *C. canephora* clonal cuttings were randomly sampled from thirteen traditional growing districts and raised to provide leaf for DNA analysis from the Coffee Research Institute (CORI), Kituza (Table 4.1). Leaves extracted for DNA were collected from clones of 16 controlled crosses and 8 elite commercials from Kituza and Kawanda

germplasm collection respectively. The controls comprised of two Ugandan wild type coffee trees, one "nganda" type from the germplasm collection and 7 representative samples from West and Central Africa. Several factors were considered while selecting study sites and sample trees. Landraces ("nganda" and "erecta" types) from traditional Robusta growing regions (Western highlands, Lake Albert and Lake Victoria Crescents, Southern drylands and South East) were exclusively selected for genetic diversity studies.

Trees that were more spreading usually with weaker upright stems that tend to bend or break with heavy crop, had small and darker green leaves were regarded as "nganda" types. The "erecta" forms were regarded as having strong stems, pale large leaves with bigger berries, although not in all cases. The two forms of Robusta are grown in mixtures and have been assumed as distinctive enough to be called varieties (Maitland, 1926; Thomas, 1935). Farmer source of planting material (wild or from fellow farmers) and tree characterization ("nganda" or "erecta") was recorded. For districts with forests, Robusta coffee trees near the wild were sampled. It was assumed that widely distributed alleles would occur in any sample. To minimize sampling error and increase chance of detecting rare alleles, many accessions (loci) were sampled. A random sample of 50 diploid individuals is adequate to have a 95% probability of detecting all alleles with a frequency of 5% or more (Mohammadi et al., 2003). To minimize chance of common maternal origin, due to the foraging behavior of insects and animals, a distance of at least five kilometers in any direction between 5 trees sampled within a locality was considered since the roads were passable. In all sites, old coffee trees were preferred on assumption that they represent land races or wild types. At least 5 tender leaves that had just attained maturity were sampled.

Table 3.1: Cultivated Robusta coffee accessions from different locations that were assessed for genetic diversity

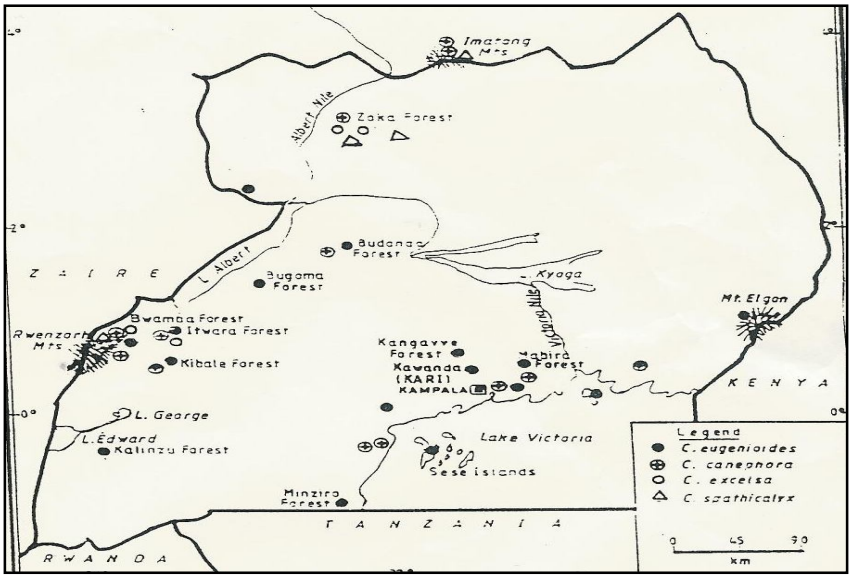
Source	Agro ecological zone	Origin	Location code	Number of accessions
District farmer collections	Western highlands	Bundibudyo	BU	11
		Kabarole	KB	31
		Kamwenge	KW	34
		Kyenjojo	KJ	37
	Lake Albert Crescent	Hoima	HM	24
		Kibale	KI	20
	Lake Victoria Crescent	Kiboga	KG	8
		Kayunga	KY	8
		Mubende	MB	13
		Mukono	MK	4
		Mpigi	MP	11
	Southern drylands	Rakai	RK	15
	South East	Jinja	JJ	3
		Kamuli	KM	12
Ugandan germplasm collections	Kawanda collections	Controlled crosses	UC	16
		Hybrids	UH	8
Controls	Uganda, West and Central Africa controls	West and Central Africa	C	4
			G	3
		Ugandan wild and "nganda" type	UW	2
			UN	1
Total				265

Key to Table 3.1: BU=Bundibudyo; HM=Hoima; JJ=Jinja; KB=Kabarole; KG=Kiboga; KI=Kibale; KJ=Kyenjojo; KM=Kamuli; KW=Kamwenge; KY=Kayunga; MB=Mubende; MK=Mukono; MP=Mpigi; RK=Rakai; UC=controlled crosses; UH=Hybrids; C, G, UW, UN=Controls (Pelezi, Mouniandougou, Nana, Inearch, Inengua, Ugandan wild and "nganda" types)

Table 3.2: Robusta coffee germplasm collections that were accessed for genetic diversity

Source	EB	UE	UN	UH	UC	Progenitors	Controls	Total
Accessions	12	19	20	6	15	7	5	84

EB=Entebbe botanical gardens; UE="erecta" types; UN="nganda" types; UH=Hybrids; Progenitors; C and G=Controls (Pelezi, Mouniandougou, Nana, Inearch, Inengua, Ugandan wild and "nganda" types).



Source: Adapted from Thomas, 1944

Figure 3.1: Map of Uganda showing the occurrence and distribution of wild coffee species, C. eugenioides, C canephora, C. excelsa and C. Spathicalyx

Note: *C. canephora* was found in the forests of Bwamoa, Itwara, Kampala Mabira, Zoka Budongo, Kibale forest, Rwenzori forest Masaka district.

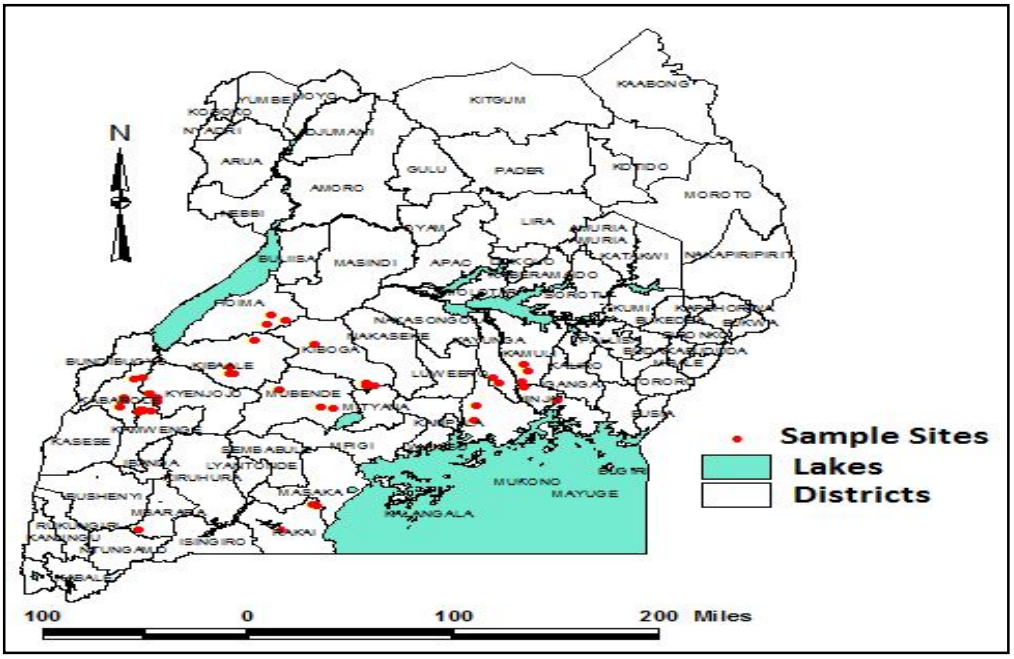


Figure 3.2: Sample sites for cultivated Robusta coffee used in genetic diversity study.

3.2.2 DNA extraction and concentrations

This work was done in CIRAD (Centre for International Agricultural Research Development) laboratory where freshly collected leaves were kept at –80 ^{o}C until grinding time. The fresh leaf samples were ground in liquid nitrogen using a pre-cooled small laboratory motor with a teaspoon of polyvinylpolypyrrolidone (PVPP) to minimize phenolic oxidation. To extract DNA, nuclear lysis with Mixed Alkytrimetylammonium Bromide (MATAB) was used according to Risterucci et al., 2000 on cocoa followed by protein precipitation polutions. The DNA concentration were determined by migrating through 0.8 to 1.0 % agarose gel against cocoa standard DNA concentrations of 2, 4, 6, 8 and 10 ng/ul. The DNA migrations in agarose gel were exposed by staining with ethidium bromide. The DNA concentrations were accordingly adjusted to concentrations of 0.5 ng/ µl.

3.2.3 SSR markers

Of the 36 markers used to evaluate Ugandan grown *C. canephora*, SSR DL011, DL013, DL025 and DL026 were derived from a bacterial artificial chromosome (BAC) library for studying sugar metabolism in coffee (Leroy et al., 2005) while 24 were from enriched library of *C. canephora* clone 126 (Dufour et al., 2002) with 9 obtained from *C. Arabica* var 'Cattura' (Combes et al., 2000; Rovelli et al., 2000). These SSRs developed in CIRAD were based on method described by Billote et al., 1999 on microsatellite-based high-density linkage map for oil palm (Elaeis guinensis Jacq.) using two heterozygous parents, tenera and a dura. Primer software (Rozen et al., 2000) was used to determine primer pairs from the flanking regions of microsatellites. Eurogentec, a South African firm, eventually synthesized the primers.

3.2.4 Polymerase Chain Reaction (PCR)

PCR amplification of micro satellite loci was done in a sealed 384 well reaction plate (Applied Bio Systems) that contained 10.0 µl final volume constituting 5.0 µl of 0.5 ng/ µl genomic DNA and 5.0 µl of PCR mix (water, 10mM tris-HCL, ph 9.0, 0.1% triton X-100, 1.5 nM of $Mgcl_2$, 0.2 pmol of primer, 0.2 nM of dCTP, dGTP, dTTP, 0.01 nM of dATP, 0.8 nCl [^{33} P]-dATP (Amersham Pharmacia, Piscataway, N.J), and 0.5 µl Taq DNA polymerase (Promega, Madison, W.S). Amplification reactions were performed in regulated temperatures for denaturation, annealing and elongation of forward and backward primers in a PTC-200 thermocycler (MJ and Appendorf Research) using a touchdown PCR procedure. The amplification cycle consisted of an initial 2 min denaturation at 94^{o}C, followed by 5 cycles of denaturation at 94 ^{o}C for 45 seconds, a 1

min primer annealing at 60 °C, with a 1 °C decrease in temperature at each cycle, 1 min 30 seconds elongation at 72 °C. Then, 30 cycles of 45 seconds at 90 °C, 1 minute at 54 °C and 1 min 30 seconds at 72 °C were performed, followed by 8 minute elongation at 72 °C.

Table 3.3: The 36 markers used to evaluate the genetic diversity of 265 Robusta coffee accessions

EMBL. Acc. No.	SSR primer	Estimated allele position		Repeat			Primer sequences (5 3')	Sequence origin	Primer origin	Species origin
		min	max	type	No.	Motif				
AJ871890	DL011	272	290	GCT, CAT	4,8	3	F:ATACATAAGCAAGCACTGA R:CAACAAATGAAATGGA	Leroy 2005	Leroy 2005	*C. canephora*, clone 126
AJ871892	DL013	277		CA, CT	6,8	2	F:AGAGGGATGTCAGCATAA R:ATTTGTGTTTGGTAGATGTG	Leroy 2005	Leroy 2005	*C. canephora*, clone 126
AJ871904	DL025	137	147	C	17	1	F:TTGTTGAGAGTGGAGGA R:CAAAGACAGTGCAGTAA	Leroy 2005	Leroy 2005	*C. canephora*, clone 126
AJ871905	DL026			A	17	1	F:CGAGACGAGCATAAGAA R:GTGGAATGAAGAATGTAG	Leroy 2005	Leroy 2005	*C. canephora*, clone 126
AJ250257	257	130	155	CA	9	2	F:GACCATTACATTTCACACAC R:GCATTTTGTTGCACACTGTA	Combes 2000	Poncet 2004	*C. canephora*, "Cattura"
AM231546	329	245	262	GT	10	2	F: ACTCAGACAAACCCTTCAAC R:GATGTTTTGCATCTATTTGG	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231551	351	308	342	GT	13	2	F:AAGGATGGCAAGTGGATTTCT R:GAGCTCTTGATTGTAGTTTCGT	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231552	355	170	212	TG	15	2	F:CTATGATGTCTTCCAACCTTCTAAC R:GGTCCAATTCTGTTTCAATTTC	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231554	358	265	321	CA	11	2	F:CATGCACTATTATGTTTGTGTTTT R:TCTCGTCATATTTACAGGTAGGTT	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231555	360	211	231	CA	10	2	F:ACAGTAGTATTTCATGCCACATCC R:ACATTTGATTGCCTCTTGACC	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231556	364	82	112	A	21	1	F:AGAAGAATGAAGACGAAACACA R:TAACGCCTGCCATCG	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231557	367	183	213	AC	12	2	F:TCAATCCCTGTATTCCTGTTT R:CTAGGCACTTAAAATCTCTATAACG	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231558	368	166	210	TG	13	2	F:CACATCTCCATCCATAACCATTT R:TCCTACCTACTTGCCTGTGCT	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231559	371	301	312	CA	9	2	F:AGACACACAAGGCAATAATCAAAC R:TCTTGAGCAGCATGGGAAC	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231560	384	263	276	AC	10	2	F:ACGCTATGACAAGGCAATGA R:TGCAGTAGTTTCACCCTTTATCC	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231563	394	137	161	TG	9	2	F:GCCGTCTCGTATCCCTCA R:GAAGCCCAGAAAGTAGTCACATAG	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231565	429	181	206	A	13	1	F:CATTCGATGCCAACAACCT R:GGGTCAACGCTTCTCCTG	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231566	442	211	245	CA	19	2	F:CGCAAATCTGAGTATCCCAAC R:TGGATCAACACTGCCCTTC	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231567	445	283	297	AC	10	2	F:CCACAGCTTGAATGACCAGA R:AATTGACCAAGTAATCACCGACT	Dufour 2001	Poncet 2007	*C. canephora*, clone 126

AM231568	456	273	313	AC	14	2	F:TGGTTGTTTTCTTCCATCAATC R:TCCAGTTTCCCACGCTCT	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231569	460	355	377	CA	11	2	F:TGCCTTCAAAATGCTCTATAACC R:GCTGATATTCTTGGATGGAGTTG	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231570	461	88	124	AC	9	2	F:CGGCTGTGACTGATGTG R:AATTGCTAAGGGTCGAGAA	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
	462	238	262				F: R:			
AM231572	471	305	339	CT	12	2	F:TTACCTCCCGGCCAGAC R:CAGGAGACCAAGACCTTAGCA	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231575	495	219	231	AC	8	2	F:CATGGATGGGAAGGCAGT R:CTTGGAAAACTTGCTGAATGTG	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM233156	501	135	181	TG	8	2	F:CACCACCATCTAATGCACCT R:CTGCACCAGCTAATTCAAGC	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AJ308753	753	283	321	CA	15	2	F:GGAGACGCAGGTGGTAGAAG R:TCGAGAAGTCTTGGGGTGTT	Rovelli 2001	Poncet 2004	*C. canephora*, "Caturra"
AJ308755	755	168	200	CA	20	2	F:CCCTCCCTCTTTCTCCTCTC R:TCTGGGTTTTCTGTGTTCTCG	Rovelli 2001	Poncet 2004	*C. canephora*, "Caturra"
AJ308774	774	225	232	CT,CA	5,7	1	GCCACAAGTTTCGTGCTTTT GGGTGTCGGTGTAGGTGTATG	Rovelli 2001	Poncet 2004	*C. canephora*, "Caturra"
AJ308779	779	117	141	TG	17	2	F:TCCCCCATCTTTTTCTTTCC R:GGGAGTGTTTTTGTGTTGCTT	Rovelli 2001	Poncet 2004	*C. canephora*, "Caturra"
AJ308782	782	134	144	GT	15	2	F:AAAGGAAAATTGTTGGCTCTGA R:TCCACATACATTTCCCAGCA	Rovelli 2001	Poncet 2004	*C. canephora*, "Caturra"
AJ308790	790	126	158	GT	21	2	F:TTTTCTGGGTTTTCTGTGTTCTC R:TAACTCTCCATTCCCGCATT	Rovelli 2001	Poncet 2004	*C. canephora*, "Caturra"
AJ308837	837	116	138	TG,GA	16, 11	2	F:CTCGCTTTCACGCTCTCTCT R:CGGTATGTTCCTCGTTCCTC	Rovelli 2001	Poncet 2004	*C. canephora*, "Caturra"
AJ308838	838	116	135	AC	9	2	F:CCCGTTGCCATCCTTACTTA R:ATACCCGATACATTTGGATACTCG	Rovelli 2001	Poncet 2004	*Coffea canephora*, "Caturra"
AM231571	463	180	260	AC	8	2	F:CATTCTTCCCACGATTCTATCTC R:GTGACTTTCGGTTGAAATACTGG	Dufour 2001	Poncet 2007	*C. canephora*, clone 126
AM231574	477	230	290	AC	16	2	F:CGAGGGTTGGGAAAAGGT R:ACCACCTGATGTTCCATTTGT	Dufour 2001	Poncet 2007	*C. canephora*, clone 126

3.2.5 Electrophoresis

A 6.5% silver stained denaturing polyacrylamide gel mixed with PVVP, 1 X Tris Borate EDTA Buffer and 8 Molar Urea was prepared and casted on a 25 centimetre plate for better resolution. A blue dye was added in the PCR products to aid visibility during electrophoresis and the DNA was denatured. A capillary comb was used to load in the PCR products and dye to the gel. A radioactively labeled 10-base pair (bp) ladder was migrated alongside investigation primers as standard using a LICOR 4300 automated

sequencer (LI-COR Biosciences, Lincoln, NE, USA). Individual polymorphism was exposed by electrophoresis of the PCR products on the denaturing polyacrylamide sequencing gels

3.2.6 Coding image bands

Since SSRs provide direct genetic information, the sequenced image bands (fragments) amplified by the primer pairs targeting polymorphic micro satellite loci were coded as alleles and designated according to fragment size (bp) as estimated by Combes et al., 2000 using SAGA Generation 2 computer program. *C. canephora* being a diploid was expected to reveal a maximum of two alleles per individual that may have differed in the gel position based on the magnitude of alleles per trait. Allele sizes were determined using allelic controls defined by Curbry et al., 2005.

3.2.7 Data analysis

Using Power Maker v.3.23 computer program, the genetic diversity (i.e genomic difference within plants) of cultivated Robusta and germplasm collection were measured using the number of distinct alleles (allelic richness) or mean alleles. Allele evenness or distribution of allelic frequencies at separate loci (allelic evenness) and genotype number that exposes different combination of alleles was accounted for by observed and expected heterozygosity as well as effective alleles. Heterozygosity values were used to express the level of genomic differences among study materials. Because Polymorphism Index Content (PIC) measures genetic diversity independent of sample sizes, was used to determine distinct allele numbers and their allelic frequencies distributed at separate loci. Genetic diversity measured allele numbers, their allelic frequencies and Nei's unbiased estimate of gene diversity (H) (Nei, 1987).

Allelobin statistical program was used to estimate the polymorphic content of each marker as quality index. Marker reliability in detecting main differences while minimizing extremes was expressed by variance values. Estimated number of generations required to have migrants exchanged between populations were derived from allelic drift figures. The minimum and maximum allele band location (range of allele size) was used to estimate the size of base pairs. Rare alleles detected by each marker were estimated as those occurring at a probability below 5% while abundant alleles occurred at 95% or above probability.

Using Darwin4 free software (developed by CIRAD), a genetic distance or dissimilarity matrix was generated with all the possible pair combination of different accessions, using Dice index (1945), equivalent to Nei et al., 1979 genetic distance.

$$SG = \frac{2n_{1,1}}{(2n_{1,1} + n_{1,0} + n_{0,1})}$$

Where $n_{1,1}$ is the number of bands shared by the individuals I and j, $n_{1,0}$ is the number of bands observed for I and missing for j, and number $n_{0,1}$ the number of bands observed for j and missing for I. This index does not take into account the information given when both I and j are missing. Nei, 1987 described genetic distance as the extent of gene differences (allelic variation) between species or populations that can be measured by some numerical quantity or any quantitative measure of genetic difference be it at the sequence level or allele frequency level calculated between individuals, population or species (Beautmont et al., 1998).

A Factorial Analysis of Dissimilarity Table (FADT) was performed and the dissimilarity matrix data of genetic distances was graphically represented as a tree (dendrogram) using un-weighted Neighbour-Joining Arithmetic Mean (AM) method as described by Saitou et al., 1987 to analyze the geographical distribution of genetic variation with 1000 bootstraps (Mohammadi et al., 2003). The 18 markers used for analysis represented most linkage groups while the 32 SSR marker data were used to compare results in other analyses.

Principal Component Analysis (PCA) was conducted using XLSTAT version 2011.2.05 statistical program. PCA linear and non-parametric pattern recognition technique was used to transform the data set of 265 genotypes of diploid nature evaluated by 18 SSRs markers to a few principal components (PCs). Tibed a large part of the variance in the data as linear combination of the original variables and explained the total inertia of the covariance matrix. PCA was used because it provides independent and balanced weighting of variance contributed by each parameter, detects genotypes placed between groups or sub groups and handles data with more accessions than parameters. The PCs described a large part of the variance in the data as a linear combination of the original variables and explained the total inertia of the covariance matrix. The PCs contained maximum possible variance of original characters in the multi dimensional space of descriptors that upon plotting gave clear relationship between two or more genotypes or characters. The total variance is denoted by Eigen values that each PC contained. Genotypes were assigned to factors based on which factor had the highest attribute variance. The first two PC were used to organize individuals as linear combinations of descriptors in two or three dimensions. The PCA of allele frequencies was performed

using pair wise comparison or correlations of accessions to derive a multi dimensional scatter plot of individuals in which the geometric distance among the individuals in the plot reflected the genetic distances among them with a high degree of accuracy (Mohammadi et al., 2003). Sub populations have fewer heterozygotes as compared to an entire population which follows Hardy Weinberg equilibrium of random mating (Nei, 1978).

Analysis of Molecular Variance (AMOVA) with allowance of less than 50% missing data was used to assess the genetic partition among and within population groups using Arlequin version 2.0 (Schneider et al., 1999). GenAlEx 6.41 statistical package (Peakall et al., 2006) was used to calculate the F statistics for between and within population differentiation (genotype frequencies). Fisher statistics F_{IS} described the degree of inbreeding by the total population (correlation of genes within individuals within populations), F_{IT} showed the total population variability, and was correlated to genes within individuals in all populations. F_{ST} measured the genetic differentiation with positive values as a correlation of different individuals in a population. F_{IS} and F_{IT} were deviations from Hardy Weinberg Equilibrium proportions within sub and total populations and were calculated as genotype frequencies. Sample differentiation test between all pairs of samples were based on haplotype frequencies.

Two mating gametes were drawn at random from each sub population to estimate the level of inbreeding within a sub population by the way of heterozygote deficiency. Values ranged from -1 to +1 where -1 implied excess and +1 a deficiency of heterozygotes in a population. Wright, 1978 suggested qualitative fixation indices for interpreting genetic differentiation as; 0.0-0.05 (little), 0.05-0.15 (moderate), 0.15-0.25

(high) while over 0.25 indicated a very great genetic differentiation. Correlation estimates for molecular distance pair wise differences among individual loci and genetic differentiation (genomic difference) significance was estimated using a proportion of corresponding bands over all populations (intra population diversity) and between each pair of populations according to (Weir et al., 1984). 1000 permutations (variations) of individuals or populations were done to test data reliability. To establish genetic diversity and variability differences among cultivated and available Robusta coffee germplasm collections, a Principal Component Analyses of dissimilarity matrix for the germplasm and farmer Robusta coffee collections were plotted and compared.

3.3 Results

A sample of allelic polymorphism at two microsatellite loci for 96 accessions *of C. canephora* is shown in Figure 3.3. All the 32 markers gave clear amplification bands that could be scored in each of the 265 samples. All the SSR markers were found polymorphic with alleles designated according to fragment size (base pairs). *C. canephora* being diploid revealed up to two alleles per accession that differed in the gel position based on the magnitude of alleles per trait.

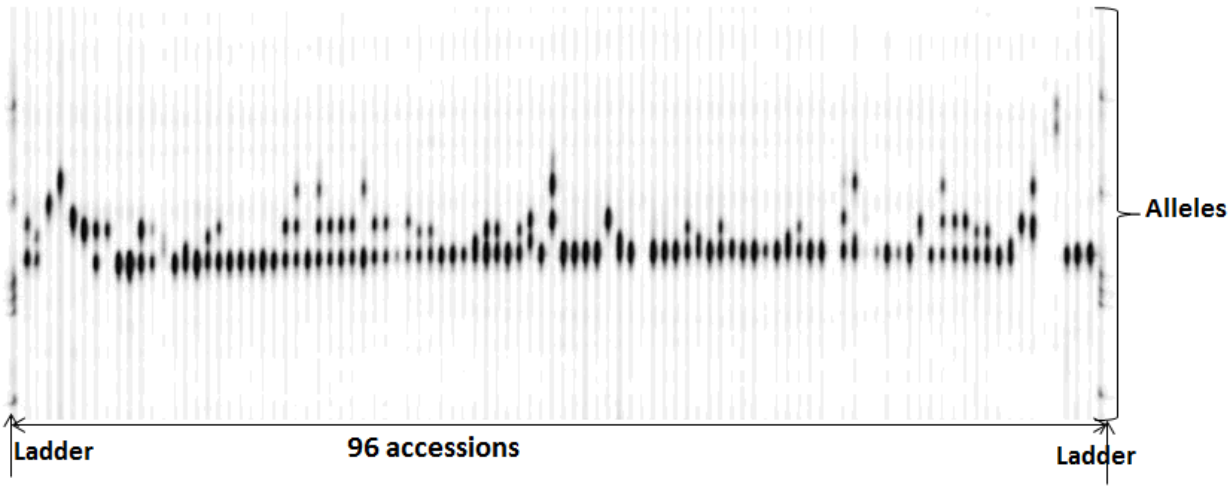


Figure 3.3: Microsatellite locus Polymorphism for 96 accessions of *C. canephora*

Key to Figure 3.3: Ladder DNA; 96accessions; alleles.

The genetic diversity of 265 *C. canephora* DNA samples as revealed by 32 different SSRs markers is shown in Table 3.4. The lowest number of 194 genotypes was recorded by SSR DL011 while marker SSR 364 evaluated 230 genotypes. Other markers evaluated a sample range of 210 to 265 genotypes. The least allele number was 7 while the highest was 30 with a mean value of 16.56. The least allele number of 7 was detected by marker DL026 followed by SSR 495 with 8 alleles. Each of marker DL025 and 456 had 30 detected alleles. Fifteen markers had allele range of 10 to 14, seven markers detected 15-20 alleles while the other six markers had 25-29 (Table 3.4). Availability for all the alleles was high as they ranged from 0.73-1.0 with a mean of 0.94. Allele 471 was detected in all study samples (availability of 1.0). Availability least scores were 0.73 and 0.79 by markers DL011 and 364 respectively. Marker 355, 495, DL013 and 782 had allele availability range of 0.87 to 0.69, while the other 26 SSRs had over 0.90.

The least different allele combination number of 11 was detected by SSR DL026 while the highest number of 116 was realized by SSR 755 with an average number of 40.88 that

reflected a high level of allele diversity. (Table 3.4) Markers that had over 80 different allele combinations included 790, 456, 779 and 461. The rest of the 22 SSRs detected less than 50 different allele combinations. The frequency of major alleles ranged from 0.11 (SSR 755) to a 0.86 score detected by marker (DL011) with an average score of 0.37. Moderate major allele frequencies of over 0.50 were realized from SSR 429, 477, 257, 384 and DL011. SSR 456,461,355, 790 and 755 major allele frequencies were less than 0.20 while 779, 351 and 471 had more than 0.20 but less 0.30. The rest of the markers major allele frequency ranged from 0.30 to 0.48 (Table 3.4). Marker DL011 detected the lowest genetic diversity value of 0.26, followed by marker 384 with a value of 0.45.

Five markers (779,456, 790, 461 and 755) detected genomic differences of above 0.90 with marker 755 having the highest assessment of 0.94. Nineteen markers revealed a genetic variability range of 0.71–0.89, while the other four were in a range of 0.57 to 0.69. A high average genomic difference value of 0.75 was obtained from genotypes in Table 3.4. The least heterozygosity was 0.01 as detected by marker DL025 with an uppermost figure of 0.90 by SSR 442 and average of 0.41. Twenty markers revealed heterozygosity of less than 0.50, seven markers had a score range of 0.50-0.60 while the other five had a score range of 0.73 to 0.82 Table 3.4. Polymorphic Index Content (PIC) had a relatively high mean value of 0.73. PIC scores of less than 0.50 were realized from SSR DL011 (0.26) and 384 (0.43) while the highest values of 0.91 and 0.94 were expressed by markers 461 and 755 respectively. The lowest PIC frequency figures were observed from SSR 341 (0.01), SSR 442 (0.08) and SSR 779 (0.09) whereas the highest had 358 (0.91), DL026 (0.96) and DL013 (0.99) (Table 3.4). Fifteen markers had a PIC

frequency of less than 0.50, eight markers ranged from 0.50-0.70 while another six had scores of over 0.70. As regards rare alleles, SSR DL011 and DL013 did not detect any. The least number of rare alleles were detected by SSR 782 (4), DL026 (4), 471 (6), 495 (7), 371 (7) and DL025 (9). The highest rare allele score was 24 from SSR 442. Over 20 rare alleles were realized from SSR 368 (20), 351 (22), 790 (22) and 442 (24).

Table 3.4: Genetic diversity of 265 Ugandan cultivated Robusta coffee cultivars as revealed by 32 SSRs markers based on Nei, 1987 parametric measure.

Marker	No. of obs.	Allele No	Availability	Genotype No	Major.allele frequency	Rare alleles	Genetic diversity	Heterozygosity	PIC	f
DL011	194	11	0.73	14	0.86	0	0.26	0.21	0.25	0.20
DL013	235	10	0.89	27	0.39	0	0.71	0.33	0.66	0.53
DL025	243	30	0.92	31	0.36	9	0.83	0.01	0.82	0.99
DL026	249	7	0.94	11	0.36	4	0.73	0.03	0.68	0.96
257	257	11	0.97	20	0.63	7	0.58	0.43	0.55	0.26
329	249	15	0.94	37	0.30	13	0.82	0.49	0.80	0.41
341	262	15	0.99	35	0.36	17	0.74	0.73	0.71	0.01
351	250	27	0.94	58	0.23	22	0.86	0.36	0.84	0.58
355	230	26	0.87	67	0.16	16	0.89	0.52	0.88	0.42
358	245	16	0.92	20	0.32	14	0.76	0.07	0.72	0.91
360	240	10	0.91	21	0.46	10	0.69	0.15	0.65	0.79
364	210	13	0.79	30	0.48	11	0.72	0.30	0.70	0.59
368	259	25	0.98	66	0.32	20	0.84	0.57	0.82	0.32
371	263	13	0.99	24	0.40	7	0.76	0.58	0.73	0.24
384	246	11	0.93	23	0.73	10	0.45	0.16	0.43	0.65
394	262	13	0.99	27	0.47	10	0.71	0.21	0.67	0.70
429	259	14	0.98	25	0.53	12	0.67	0.46	0.64	0.32
442	257	28	0.97	51	0.31	24	0.84	0.90	0.82	-0.08
445	262	10	0.99	23	0.42	10	0.71	0.21	0.67	0.70
456	261	30	0.98	80	0.18	19	0.90	0.75	0.90	0.17
461	264	27	1.00	96	0.16	15	0.92	0.77	0.91	0.16
471	265	14	1.00	41	0.28	6	0.82	0.46	0.80	0.44
477	262	10	0.99	14	0.55	17	0.57	0.10	0.50	0.83
495	230	8	0.87	20	0.39	7	0.76	0.47	0.72	0.38
501	258	12	0.97	29	0.39	13	0.75	0.50	0.72	0.34
753	257	10	0.97	24	0.37	15	0.72	0.34	0.68	0.53
755	259	29	0.98	116	0.11	16	0.94	0.59	0.94	0.37
779	261	20	0.98	89	0.22	13	0.90	0.82	0.89	0.09
782	236	16	0.89	27	0.32	4	0.77	0.24	0.73	0.69
790	260	19	0.98	80	0.13	22	0.91	0.60	0.90	0.34
837	261	19	0.98	55	0.26	11	0.86	0.51	0.85	0.42
838	251	11	0.95	27	0.41	11	0.75	0.12	0.72	0.85
Mean	**249.9**	**16.56**	**0.94**	**40.88**	**0.37**	**12.83**	**0.75**	**0.41**	**0.73**	**0.46**

Except with rare alleles, the number of alleles was significantly and positively related with all the genetic diversity measures (Table 3.5). Allele availability had significant and

positive relationships with the number of genotypes and rear alleles whereas major allele frequency significantly and positively associated with only rare alleles. The number of genotypes was positively and significantly associated with genetic diversity estimates.

Table 3.5: Tukey's pairwise comparison of genetic diversity of Ugandan farmer Robusta coffee assessed by 32 SSRs markers

	No.of obs	allele no	Avail	Genotype no	Major allele freq	rare Alleles	Genetic diversity	Heterozy	PIC	Freq
No.of obs.	1									
allele no	126.5*****	1								
Availability	134.9*****	8.47*****	1							
Gtype no	113.3*****	13.2*****	21.64*****	1						
M allele F	135.3*****	8.78*	0.31	21.95*****	1					
rare alleles	128.5*****	2.02	6.45*****	15.2******	6.76*****	1				
G. diversity	135*****	8.57*****	0.10	21.75*****	0.208	6.55*****	1			
Heterozy	135.2*	8.76*****	0.29	21.94*****	0.02	6.74*****	0.19	1		
PIC	135.1*	8.58*****	0.12	21.76*****	0.19	6.56*****	0.01	0.18	1	
Frequency	135.2*****	8.72*****	0.26	21.9*****	0.06	6.7*	0.15	0.04	0.14	1

* significant at 0.0002 levels of probability

Key to Table 3.5: No.of obs=number of observations; Avail= availability; Gtype no=genotype number; M allele F=major allele frequency; G diversity= genetic diversity; Heterozy=Heterozygosity; PIC=polymorphic information content; Freq= frequency

Table 3.6 shows the quality of the 18 selected SSRs molecular markers that were used to assess the genetic diversity of farmer Robusta coffee, their specific marker allele range and location, plus allele abundance and scarcity. The efficiency of the selected 18 markers to detect polymorphism (quality index) was relatively high as all the SSRs had scores of 0.50 and below. Quality index values ranged from 0.0002 for marker 364 to 0.5 for marker 442. Ten SSR (DL026, 364, 461, 790, 429, 384, 471, 368, 358 and 355) had high efficiency in detecting polymorphism as their quality indices were less than 0.02.

Four other SSR (445, 837, 394 and 456) had quality indices value range of 0.21-0.40 and another four SSR (501, 477, 753 and 442) had score range 0.41-0.50. The study showed that the SSRs reliability represented by average minimum variance were in the short range of 0.00-0.22, reflecting a high level of reliability as high values reflect extreme cases. Six markers (DL026, 364, 384, 429, 461 and 790) had 0.00 values while another six (358, 368, 471, 355, 445 and 837) had values ranging from 0.01-0.10 score. SSR 394, 456, 442, 501, 477 and 753 scores were in the range of 0.11-0.22. As per allelic drift, 12 SSR (355, 358, 364, 368, 384, 429, 445, 456, 461, 471 and 790) had 0.0 values. Allelic drift values for other markers were as follows; 394 (0.02), 501 (0.04), 753 (0.04), 837 (0.04), 477 (0.1), 442 (0.14). Observed allelic gene flow among cultivated Robusta coffee landraces were low with a single migrant allele introduced or exchanged at least after 7 generations. As per marker range of variability, the least allele range of 12 was from SSR DL026 while the largest of 60 was from SSR 355. Small marker ranges reflect small size alleles while larger marker ranges are associated with bigger size alleles. Other markers that detected high allele variability included, 442 (58) and 368 (54). The other 13 SSR had an allele range of 20-48. Overall, allele range of 12-60 reflected a high level of variability among cultivated Robusta coffee. Abundant allele frequency of 67% was detected by marker 477 at locus 264. Other abundant allele frequencies of above 50% were revealed by marker 394 (51.15%), 445 (53.45%) and DL026 (55.88%). Abundant allele frequencies of less than 25% were revealed by marker 456 (10.92%), 790 (14.65%) and 355 (18.03%).

Table 3.6: Quality of the 18 selected markers used in assessing Ugandan cultivated Robusta diversity

SSR marker	Quality index	Av. Min variance	Allelic drift (delta)	Locus					
				Min allele	Max allele	Allele range	Abundant allele	Abundant allele freq	Rare allele (< 5%)
DL026	0.0005	0.00	0.00	133	145	12	135	55.88	139,141,143,145 **(4)**
355	0.15	0.02	0.00	155	215	60	197	18.03	155,157,159,161,163,167,169,171,173,175,177,179,181,183,185,187,203,205,209,213,215 **(21**)
358	0.11	0.01	0.00	267	309	42	293	42.86	267,269,271,279,281,285,287,297,303,305,309 **(11)**
364	0.0002	0.00	0.00	99	114	15	106	46.10	99,101,105,109,110,111,112,114 **(8)**
368	0.10	0.01	0.00	162	216	54	170	31.03	162,164,172,182,184,186,188,190,192,196,198,200,202,206,208,216 **(16**)
384	0.06	0.00	0.00	262	282	20	274	49.20	262,264,266,268,276,278,280,282 **(8)**
394	0.341	0.11	0.02	142	184	42	148	51.15	142,144,150,152,156,158,160,164 **(8**)
429	0.0005	0.00	0.00	180	208	28	182	31.47	180,183,186,188,190,192,195,199,200,204,206,208 **(12**)
442	0.50	0.17	0.14	200	258	58	204	37.98	214,216,218,226,228,230,232,234,236,238,240,242,244,246,248,250,252,254,256,258 **(20**)
445	0.21	0.04	0.00	284	308	24	294	53.45	284,286,290,298,300,302,308 **(7)**
456	0.40	0.15	0.00	251	321	30	307	10.92	261,263,265,267,271,273,277,281,289,291,297,299,301,305,309,311,313,321 **(18)**
461	0.0002	0.00	0.00	81	129	48	105	28.27	81,83,97,99,101,107,111,113,117,119,121,123,125,127,129 **(15)**
471	0.08	0.01	0.00	307	343	36	313	25.10	307,309,323,325,327,329,331,337,343 **(9)**
477	0.46	0.18	0.10	236	282	46	264	67.05	236,242,246,268,270,272,276,2829 **(8**)
501	0.41	0.17	0.04	146	178	32	148	43.02	156,158,160,162,170,174,178
753	0.48	0.22	0.04	284	322	38	316	36.02	284,304,306,308,310,312,322
837	0.30	0.09	0.04	118	142	24	130	46.65	118,120,122,134,136,138,140,142 **(8)**
790	0.0002	0.00	0.00	120	162	42	138	14.65	120,128,144,148,150,152,154,156,160,162 **(10)**

The 18 SSR markers used to assess the genetic diversity of 265 cultivated Robusta coffee genotypes were polymorphic (Table 3.7). SSR DL026 gave the least allele number of 7, 364 (12) while 35 was recorded by SSR 456 as the highest. Most alleles were observed with marker 355 (35) and 456 (35). Low scores for major allele frequency was from SSR 456 (0.10), 790 (0.15) and 355 (0.18). It therefore implies that as much as marker 355 and 456 had many alleles, they were not of major type. Marker 384 (0.49), 394 (0.50) and DL026 (0.56) had notably moderate major allele frequencies. SSR markers DL026 (11), 358 (23) and 364 (23) had the lowest values for different allele combinations (genotype numbers) whereas higher values were attained by SSR 790 (69), 355 (71) and 456 (92). DNA genomic difference (genetic diversity) was lowest with markers DL026 (0.61) and 445 (0.63) and highest with SSR 355 (0.89), 790 (0.89) and 456 (0.94). The lowest accession variability (heterozygosity) was revealed by SSRs DL026 (0.02), 358 (0.11), 384 (0.14) and 394 (0.19) whereas high values were achieved from 790 (0.67), 456 (0.72) and 442 (0.82). PIC values were also low in marker DL026 (0.55), 445 (0.58), 384 (0.63) and high with SSR 355 (0.88), 790 (0.88) and 456 (0.93). From results it was noted that SSRs markers that had few alleles also had high major allele frequency. Genotypes with low different allele combinations tended to have low genome difference, genomic variability and PIC score and vise versa. The PIC values were all high ranging from 0.55 to 0.93.

Table 3.7: Mean diversity values for Ugandan cultivated Robusta coffee using 18 selected markers

Markers	Sample size	Allele no.	Major allele freq	Genotype no.	Gene diversity	Heterozy gosity	PIC
DL026	265	7	0.56	11	0.61	0.02	0.55
355	265	35	0.18	71	0.89	0.53	0.88
358	265	16	0.43	23	0.73	0.11	0.70
364	265	12	0.46	23	0.72	0.26	0.69
368	265	28	0.31	62	0.86	0.59	0.85
384	265	12	0.49	24	0.67	0.14	0.63
394	265	14	0.50	26	0.69	0.19	0.67
429	265	17	0.31	34	0.79	0.49	0.76
442	265	32	0.38	44	0.80	0.82	0.78
445	265	13	0.53	25	0.63	0.21	0.58
456	265	35	0.10	92	0.94	0.72	0.93
461	265	19	0.28	59	0.84	0.52	0.82
471	265	17	0.25	45	0.85	0.50	0.83
477	265	13	0.37	24	0.74	0.08	0.70
501	265	16	0.42	34	0.76	0.54	0.73
753	265	13	0.29	35	0.81	0.38	0.79
837	265	18	0.43	45	0.77	0.37	0.75
790	265	18	0.15	69	0.89	0.67	0.88
Mean	**265**	**19**	**0.36**	**41**	**0.78**	**0.40**	**0.75**

Key to Table 3.7: no=number; freq=frequency; PIC=polymorphic information content.

There were significantly positive relationships between the number of alleles and genotypes with major allele frequency, gene diversity heterozygosity and PIC values (Table 3.8). Major allele frequency had a significant positive relation with the number of genotypes.

Table 3.8: Tukey pairwise comparison of mean diversity values for Ugandan cultivated Robusta coffee using 18 selected markers

	Allele	Major allele Freq	Genotypes	Gene diversity	Heterozy	PIC
Allele	1					
Major allele Freq	8.35*****	1				
Genotypes	10.32*****	18.57*****	1			
Gene diversity	8.06*****	0.20	18.38*****	1		
Heterozy	8.23*****	0.02	18.55*****	0.17	1	
PIC	8.07*****	0.18	18.39*****	0.02	0.16	1

* Significant at 0.0001 level of probability; Key to Table 3.8: Refer to 3.5

The mean intra population molecular diversity parameters calculated according to Nei, 1987 as assessed by 18 loci at up to 50% level of missing data is presented in Table 3.9. Kyenjojo population had the most total gene copies of 74. The lowest gene copy numbers were found in accessions from Jinja (6), Guinean (6), Ugandan (6) and Congolese (8) controls and Mukono (8) while the remaining others had 16-68 range. Kamuli population had the lowest usable loci of 4.0 and the least polymorphic loci value of 4.0. Jinja had the most usable loci of 17.0. Kibale population despite having the highest number of polymorphic loci of 16 had the lowest gene diversity over the loci of 0.50. Low genetic diversity over loci of 0.53 was observed from Ugandan control, Jinja and Kabarole populations. Gene diversity over loci was highest in Kiboga (0.78), hybrids (0.72) and Kamwenge (0.71). The highest number of usable gene copies was recorded in Kyenjojo (70), Kamwenge (64.11) and Kabarole (57.44) and the lowest in Guinean control (5.33), Ugandan control (5.67) and Jinja (5.67). Most alleles were high in Kamwenge (9.56), Kyenjojo (7.94), Hoima (6.39), Kibale (6.22) and Mubende (6.06). The least allele values came from populations with the least total gene copies; Jinja (2.44), Ugandan control (2.56), Guinea (2.61) and Mukono (2.94). Observed heterozygosity (Ho) was less than expected heterozygosity (He) in all populations. Observed

heterozygosity range was 0.23 (Guinea population) to 0.50 in the hybrid. Expected heterozygozity was highest in the Congolese (0.73) and Kamwenge (0.72) while other population values ranged from 0.50 (Jinja) to 0.70 (Kiboga).

Table 3.9 Mean intra population molecular diversity parameters of 265 cultivated Ugandan *C. canephora* calculated according to Nei, 1987

Pop	Total gene copies	Usable loci	Poly loci	Gene diversity over loci	Usable gene copies	Alleles	Heterozygosity		Significantly different				
							Obs (Ho)	Exp (He)	Gene diversity over loci	Gene copies	Alleles	Obs (Ho)	Exp (He)
BU	22	14	14	0.61	20.11	5.11	0.40	0.56	0.33	5.23	2.96	0.31	0.31
C	8	16	15	0.70	7.33	3.72	0.44	0.73	0.40	1.89	1.41	0.33	0.25
G	6	13	13	0.67	5.33	2.61	0.23	0.58	0.41	1.16	1.16	0.32	0.32
UG	6	15	12	0.53	5.67	2.56	0.42	0.55	0.33	0.75	1.17	0.34	0.28
HM	48	15	14	0.59	44.56	6.39	0.39	0.58	0.31	10.93	3.17	0.26	0.25
JJ	6	17	13	0.53	5.67	2.44	0.33	0.50	0.33	1.37	1.30	0.35	0.33
KB	62	14	14	0.53	57.44	5.78	0.28	0.52	0.28	14.11	3.08	0.23	0.24
KG	16	7	7	0.78	14.33	4.94	0.38	0.70	0.44	1.92	1.96	0.25	0.21
KI	40	16	16	0.50	38.67	6.22	0.38	0.56	0.26	2.21	3.08	0.28	0.27
KJ	74	12	12	0.68	70.00	7.94	0.37	0.67	0.36	5.96	3.72	0.25	0.18
KM	24	4	4	0.63	20.78	5.44	0.47	0.66	0.39	2.76	1.67	0.28	0.16
KW	68	11	11	0.71	64.11	9.56	0.42	0.72	0.37	5.68	3.83	0.26	0.18
KY	16	14	14	0.61	14.89	4.39	0.47	0.59	0.33	2.33	2.22	0.31	0.20
MB	26	8	8	0.68	23.78	6.06	0.41	0.66	0.38	2.49	2.59	0.30	0.21
MK	8	15	12	0.56	7.56	2.94	0.47	0.60	0.33	1.07	0.33	1.18	0.29
MP	22	13	13	0.62	21.00	5.50	0.46	0.64	0.33	2.03	2.19	0.29	0.20
RK	30	11	11	0.61	27.78	5.67	0.44	0.62	0.33	4.67	2.36	0.28	0.20
UC	32	12	12	0.62	29.44	5.44	0.41	0.60	0.33	6.86	2.36	0.29	0.23
UH	16	5	5	0.72	13.89	4.72	0.50	0.68	0.43	1.82	1.94	0.31	0.17

Three major diversity groups were formed from Ugandan cultivated Robusta coffee (Figure 3.4). From the tree, individual accessions were brought together resulting into three larger genetic diversity groups. The controls from West and Central Africa formed a group of their own while the controlled hybrids located in one group and hybrids in another different group. One group was dominated by genotypes from Kabarole, Bundibudyo (Western highlands region) and Hoima (Lake Albert Crescent region) located in Western Uganda. A second group comprised genotypes from adjacent districts of Kyenjojo (Western highlands), Kiboga (Lake Victoria Crescent bordering Lake Albert Crescent region) and Kamuli at the far South East. The third group constituted a mixture of accessions from different locations; Kayunga, Mpigi, Mubende, Rakai, Mukono from Lake Victoria Crescent and Kamwenge from Western highlands.

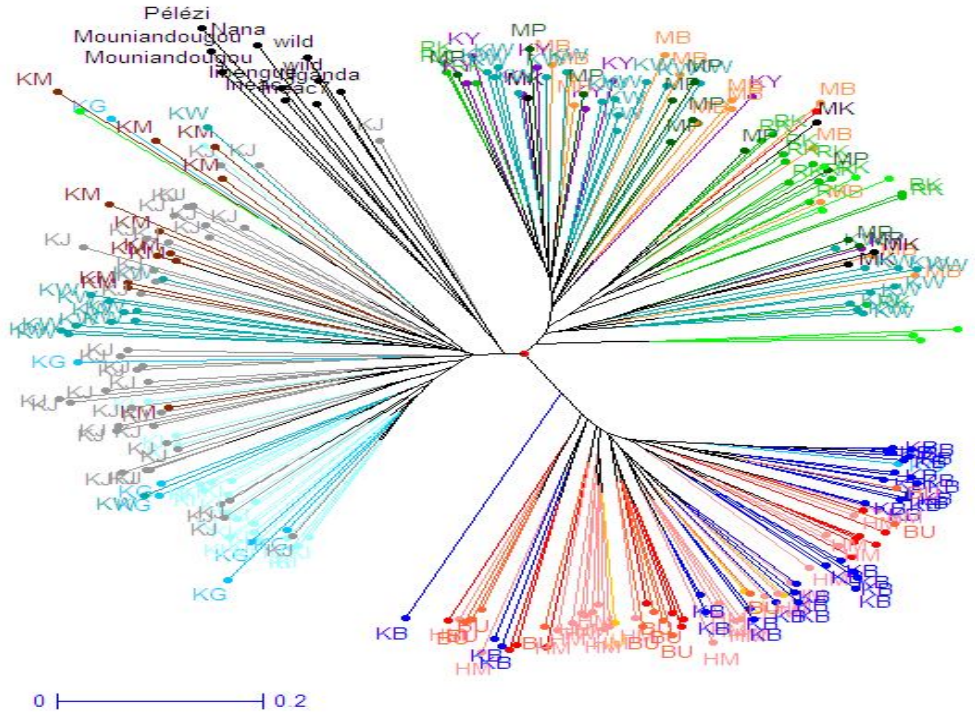


Figure 3.4: Neighbour Joining tree derived from Un-weighted Pair Group Arithmetic Mean using Factorial Analysis of Dissimilarity matrix for 265 genotype DNA with 18 SSR markers; Key: site codes as in Table 3.1

Geometric distribution among plotted accessions on X and Y axes reflected genetic distances of aggregated genetically similar individuals (Figure 3.5). Controlled crosses were grouped with genotypes from Kabarole, Bundibudyo and Hoima. The hybrids were mainly distributed in one group located on the negative side of the X axis. The controls were located mainly between the two groups on the negative side of the X axis. The distance between aggregated groups seemed similar.

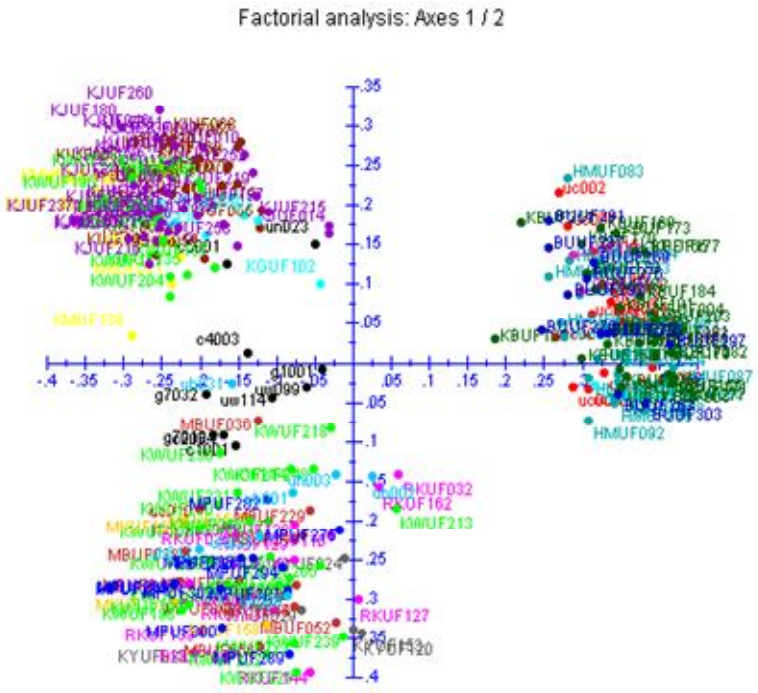


Figure 3.5: Principal Component Analysis of dissimilarity matrix for Robusta coffee genotypes from farmer fields and germplasm collections using 18 SSR markers

Figure 3.6 shows the genetic representation of cultivated Robusta coffee diversity groups using a dendrogramme. Except for UC010 (L/2/7Rep1Tree4), the controlled crosses were located in one genetic diversity group while the hybrids clustered in another except for UH007 (258^{s}/24(0). The controls constituted a sub group of another main diversity group.

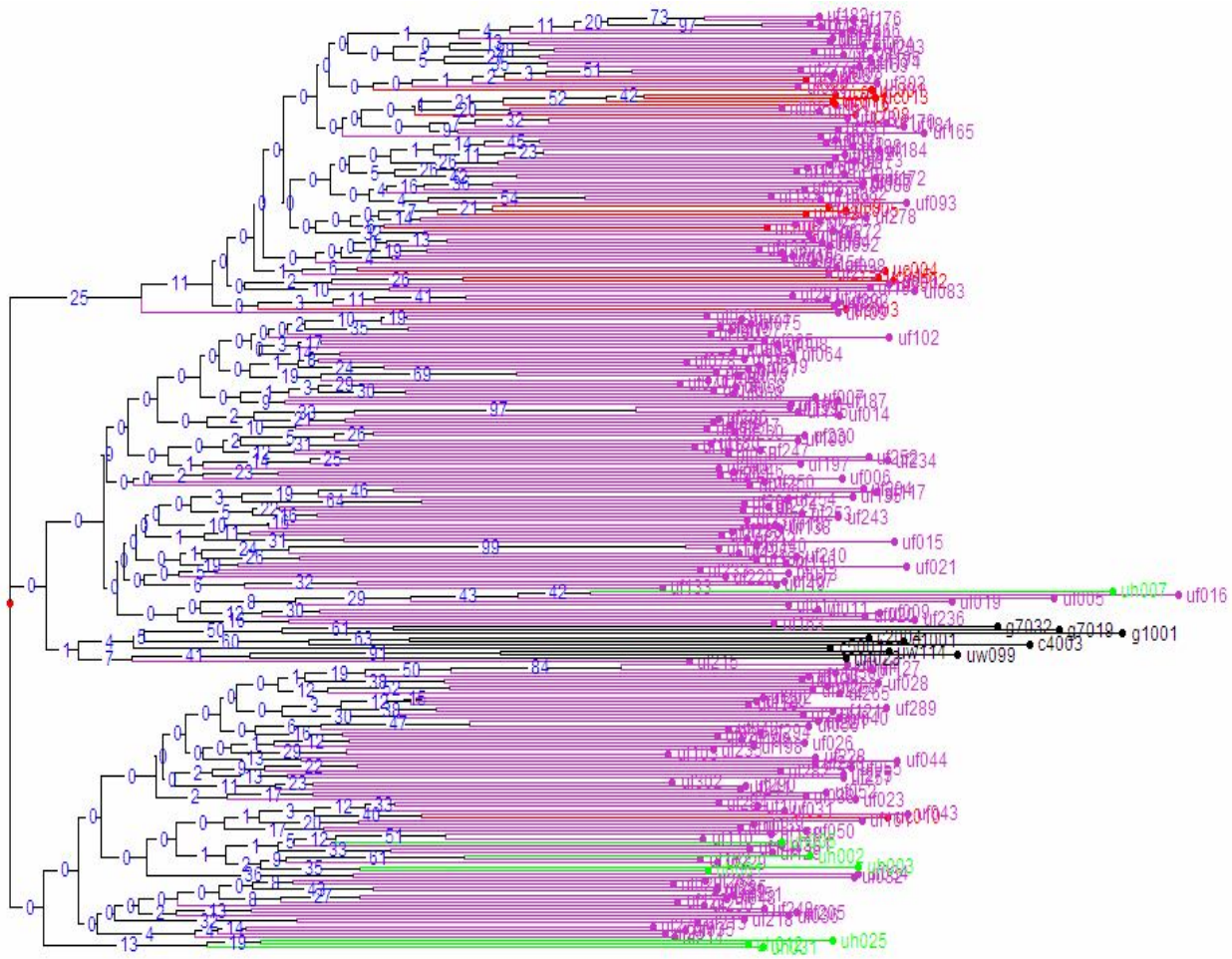


Figure 3.6: Dendrogram of Un-weighted Neighbour Joining Arithmetic Mean from Factorial Analysis of Dissimilarity table for DNA analysis with 18 SSR

Key to Figure 3.6: UF= Farmer landraces; UH=Hybrid; UC=Controlled crosses; UN, UW, G, C=Controls

Analysis of molecular variance showed most significant differences were within population group individuals (28.92%) and within individuals (54.05%) as in Table 3.10. There were no significantly different populations (significance level=0.05). Differences among populations and among individuals within populations were minimal. Inbreeding index (F_{IS}) value was as low as 0.35, implying Robusta coffee was dominantly out crossing. The breeding index for all cultivated Robusta coffee genotypes was below average (0.46), pointing out that crossing of geographically distant genotypes was limited, close to average.

Table 3.10: Molecular Analysis of Variance (AMOVA) for cultivated Robusta coffee accessions

Source of variation	d.f	Sums of squares	Variance components	Percentage of variation	F_{IS}	F_{IT}	F_{ST}
Among populations	18	434.68	0.72	17.03a	0.35	0.46	0.17
Among individuals within populations	246	1161.60	1.22	28.92b			
Within individuals	265	604.50	2.28	54.05c			
Within populations	511	1766.10	3.46	82.97			
Total	529	2200.78	4.22				

The total expected heterozygosity of loci ranged from 0.67 to 0.94, of which values could be regarded as high (Table 3.11). The least total expected heterozygosity was observed with SSR marker DL026 (0.67) and marker 445 (0.71) while SSR 355 (0.92 and 356 (0.84) recorded the highest scores. Low rating of less than 0.4 mean expected heterozygosity across populations was recorded by marker DL026 (0.31), marker 445 (0.36) and marker 384 (0.40). Scores of over 0.70 mean expected heterozygosity were achieved by SSR marker 368 (0.71), marker 456 (0.73), marker 355 (0.76) and marker 790 (0.77). Average observed heterozygosity across populations of less than 0.01 was notably by SSR DL026 (0.01), marker 358 (0.07) and marker 477 (0.06) while the highest values were attained by SSR 456 (0.70) and marker 442 (0.77). SSRs markers that detected the highest *C. canephora* incompatibility nature were marker 442 (-0.17) and marker 456 (0.05). Ten markers had F_{IS} values that ranged from 0.15-0.38 while another six markers had values of 0.58 and above with SSR DL026 having the highest inbreeding score of 0.96. Inbreeding index for total samples (F_{IT}) ranged from 0.05 (SSR 442) to 0.98 (DL026) with an average F_{IT} value of 0.55±0.06. F_{IT} values were slightly higher than F_{IS} scores although the weightings across markers were similar. Markers DL026, 358, 477, 384 and 394 had consistently high values of 0.63 and above for both F_{IS} and F_{IT}. Population differentiation index (F_{ST}) was low in

markers 790 (0.13), 355 (0.17), 442 (0.19) and highest in marker 445 (0.60) and marker DL026 (0.53). Average population differentiation index was 0.31±0.03. Gene flow (Nm) ranged from 0.22 (marker DL026) to 1.67 (marker 790).

Table 3.11 Fisher statistics and estimates of inbreeding, (10,000 permutations with significance tests of 1023 permutations at P=0.05)

SSR	H_t	Mean H_e	Mean H_0	F_{IS}	F_{IT}	F_{ST}	Nm
DL026	0.67	0.31	0.01	0.96	0.98	0.53	0.22
355	0.92	0.76	0.50	0.34	0.45	0.17	1.24
358	0.81	0.48	0.07	0.84	0.91	0.41	0.36
364	0.77	0.45	0.19	0.58	0.76	0.42	0.34
368	0.88	0.71	0.52	0.27	0.42	0.20	0.99
384	0.72	0.40	0.14	0.64	0.80	0.44	0.31
394	0.74	0.45	0.17	0.63	0.77	0.38	0.40
429	0.83	0.59	0.50	0.15	0.39	0.29	0.62
442	0.81	0.66	0.77	-0.17	0.05	0.19	1.07
445	0.71	0.36	0.23	0.35	0.68	0.50	0.25
456	0.94	0.73	0.70	0.05	0.26	0.22	0.89
461	0.86	0.68	0.50	0.27	0.42	0.21	0.95
471	0.85	0.67	0.49	0.27	0.43	0.21	0.92
477	0.76	0.45	0.08	0.82	0.89	0.41	0.36
501	0.81	0.57	0.49	0.15	0.40	0.30	0.59
753	0.84	0.64	0.40	0.38	0.53	0.24	0.80
837	0.82	0.59	0.43	0.28	0.48	0.28	0.65
790	0.89	0.77	0.60	0.23	0.33	0.13	1.65
Mean				0.39	0.55	0.31	0.70
SE				0.07	0.06	0.03	0.09

Key: Mean H_e=average H_e across the populations; Mean H_0=Average H_0 across the populations; F_{IS}=Inbreeding Index; F_{IT}=Inbreeding Index for total samples; F_{ST}=Population differentiation Index; Nm=Gene flow; H_t=Total Expected Heterozygosity.

Population differentiation indices (F_{ST}) and inbreeding index (F_{IS}) indices after 10,000 permutations are shown in Table 3.12. Average population differentiation index was 0.18. Except for Kamwenge populations that had 0.17 rating, the rest of the study populations had either 0.18 or 0.19 differentiation indices. The highest inbreeding index value of 0.81 was achieved by Guinea control population while the Ugandan control population had the least value of 0.02. The rest of the populations had inbreeding indices that ranged from 0.20 to 0.49. In 12 districts, there was no likelihood that randomly observed inbreeding indices

would differ. At P=0.05, no significant difference was realized between randomly observed inbreeding indices for the Guinean and Ugandan controls as well as collections from Jinja and Mukono locations. There were significant differences among randomly observed inbreeding indices for Congolese types, hybrids and genotypes from Kayunga were apparent.

Table 3.12: Population inbreeding, differentiation and gene flow indices (10100 permutations with significance tests of 1023 permutations)

No.	**Populations**	**F_{ST}**	**F_{IS}**	**P(random FIS >=observed FIS**
1	Bundubudyo	0.18	0.29	0
2	Congolese	0.18	0.49	0.01
3	Guinean	0.19	0.81	0.06 ns
4	Uganda	0.18	0.02	0.32 ns
5	Hoima	0.18	0.32	0
6	Jinja	0.19	0.33	0.12 ns
7	Kabarole	0.18	0.44	0
8	Kiboga	0.18	0.46	0
9	Kibale	0.19	0.26	0
10	Kyenjojo	0.18	0.38	0
11	Kamuli	0.18	0.22	0
12	Kamwenge	0.17	0.43	0
13	Kayunga	0.18	0.24	0.0008
14	Mubende	0.18	0.35	0
15	Mukono	0.18	0.20	0.13 ns
16	Mpigi	0.18	0.30	0
17	Rakai	0.18	0.31	0
18	Crosses	0.18	0.26	0
19	Hybrid	0.18	0.22	0.01
Mean		**0.18**	**0.33**	

Key: ns=not significant while the rest were significant.

Sample differentiation test between all pairs of samples based on haplotype frequencies revealed negative values among populations as shown in Table 3.13. Negative haplotype frequency values implied the populations were segregating and heterozygotes or genotypes had evolved in different locations.

Table 3.13 Genetic differentiation among populations (significance level=0.05)

	BU	CO	GU	UG	HM	JJ	KB	KG	KI	KJ	KM	KW	KY	MB	MK	MP	RK	UC	UH
BU																			
C	-																		
G	-	-																	
UG	-	-	-																
HM	-	-	-	-															
JJ	-	-	-	-	-														
KB	-	-	-	-	-	-													
KG	-	-	-	-	-	-	-												
KI	-	-	-	-	-	-	-	-											
KJ	-	-	-	-	-	-	-	-	-										
KM	-	-	-	-	-	-	-	-	-	-									
KW	-	-	-	-	-	-	-	-	-	-	-								
KY	-	-	-	-	-	-	-	-	-	-	-	-							
MB	-	-	-	-	-	-	-	-	-	-	-	-	-						
MK	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
MP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
RK	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
UC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
UH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

From the PCA Figure 3.7a, genotypes from the germplasm collection were widely distributed in all sections without consolidating into any single group. Controlled crosses were grouped with genotypes from Kabarole, Bundibudyo and Hoima (Figure 3.7b). The hybrids were distributed among the two other groups located on the negative side of the X axis. The controls were located mainly between the two groups on the negative side of the X axis.

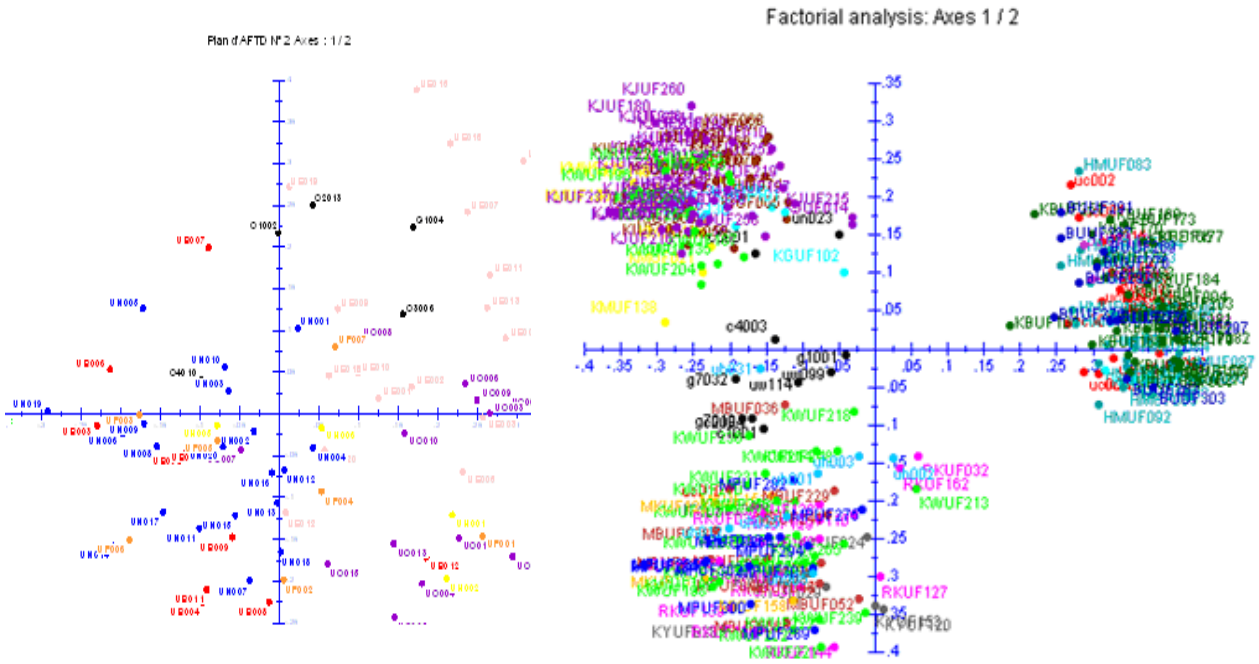


Figure 3.7 (a, b): Principal Component Analyses of dissimilarity matrix for (a) germplasm collections and (b) cultivated Robusta coffee

Key: EB=Botanical gardens Entebbe (12); UE="erecta" (19); UN="nganda" (20); UH=Hybrids (6); UC=Controlled crosses (15); Progenitors (parents =7); C and G=Check samples(Pelezi, Mouniandougou, Nana, Inearch, Inengua = 5).

Genetic diversity values for cultivated and conserved germplasm are shown in Table 3.14. Most cultivated collections were from Kyenjojo (37), Kamwenge (34), Kabarole (31) while the least were from Jinja (3), Mukono (4) and controls (1-4) (Table 3.14). Germplasm population numbers ranged from 1-4. Number of different alleles ranged from 1.50 ("nganda" control) to 10.06 (Kamwenge) with a grand mean of 5.25. The germplasm collection allele range was 4.05 for the controls and 17.75 for "erecta". Cultivated collections had more effective alleles than the germplasm collection with respective ranges of 2.14 (Jinja) to 4.88 (Kamwenge) and 2.74 (hybrids) to 3.49 ("nganda") as seen in Table 3.12. Shannon Diversity Index (I) values were highest in populations of Kamwenge (1.71) and Kyenjojo (1.55), reflecting *C. canephora* higher diversity than the "nganda" (0.35), Ugandan wild (0.48), Guinean (0.93) controls and Mukono (0.93) with lower values. Germplasm

collections had higher heterozygosity values in the range of 0.47-0.66 (observed), 0.58-0.69 (expected) and 0.65-0.80 (unbiased expected). The lower heterozygosity ranges for cultivated Robusta coffee were 0.20-0.50 (H_o), 0.25-0.73 (H_e) and 0.43-0.74 (UH_e). Population with high fixation indices (F) were Kiboga (0.52), Kabarole (0.50), Mubende (0.49) , Kamwenge (0.46) while lower values were among progenitors (0.03) and Entebbe botanical garden collections (0.17). Rare allele numbers were high among Congolese (10) and Guinean (7) controls and accessions from Kamwenge (10) and Kyenjojo (8). Rare alleles were more in the "nganda" (5-6) than "erecta" (1) while collections from Entebbe botanical gardens had 5.

Table 3.14: Comparison of mean population heterozygosity over loci for 265 cultivated and 84 germplasm collection accessions

Pop	**N**	**Na**	**Ne**	**I**	**Ho**	**He**	**UHe**	**F**	**Av Pa**	**Pa loci**	**Loci with rare alleles**
BU	11	5.33	3.23	1.17	0.40	0.55	0.58	0.33	0.2	3	355, 471, 477 **(3)**
C	4	3.83	2.97	1.11	0.36	0.59	0.67	0.39	0.9	10	355, 358, 364, 368, 394, 442, 456, 471, 501, 753 **(10)**
G	3	2.89	2.61	0.93	0.20	0.56	0.67	0.68	0.5	7	355,358,364,456,477, 501, 753 **(7)**
HM	24	6.67	3.19	1.24	0.39	0.57	0.59	0.34	0.4	4	355, 442, 456, 753 **(4)**
JJ	3	2.50	2.14	0.71	0.33	0.42	0.50	0.26	nd		nd
KB	31	6.06	2.76	1.11	0.28	0.52	0.53	0.50	0.1	2	456, 790 **(2)**
KG	8	5.56	3.85	1.43	0.35	0.69	0.74	0.52	0.1	2	368, 477 **(2)**
KI	20	6.61	3.32	1.25	0.36	0.55	0.57	0.34	0.2	3	442, 456, 471 **(3)**
KJ	37	8.50	4.12	1.55	0.36	0.69	0.69	0.47	0.6	8	355, 358, 442, 364, 368, 429, 477, DL026 **(8)**
KM	12	6.22	3.63	1.44	0.40	0.68	0.71	0.43	0.3	4	384, 442, 445, DL026 **(4)**
KW	34	10.06	4.88	1.71	0.40	0.73	0.74	0.46	0.8	10	355, 358, 368, 384, 394, 429, 442, 456, 471, 790 **(10)**
KY	8	4.61	3.03	1.16	0.44	0.59	0.63	0.32	nd		nd
MB	13	6.61	3.97	1.45	0.38	0.67	0.70	0.49	0.2	4	355, 368, 429, 445 **(4)**
MK	4	3.11	2.58	0.93	0.43	0.53	0.60	0.19	nd		nd
MP	11	5.78	3.39	1.32	0.43	0.62	0.65	0.35	0.2	3	358, 445, 837 **(3)**
RK	15	6.06	3.08	1.29	0.39	0.61	0.63	0.41	0.2	3	355, 477, 837 **(3)**
UC	16	5.78	3.15	1.24	0.40	0.60	0.62	0.43	0.1	2	358, 461 **(2)**
UH	8	5.44	3.66	1.41	0.43	0.69	0.74	0.40	0.2	4	358, 442, 477, 790 **(4)**
UN=C	1	1.50	1.50	0.35	0.50	0.25	0.50	-1.00	0.1	2	355 368 **(2)**
UW=C	2	1.83	1.67	0.48	0.31	0.32	0.43	0.01	0.1	2	355 429 **(2)**
Grand av.	**13**	**5.25**	**3.14**	**1.16**	**0.38**	**0.57**	**0.62**	**0.36**			
C	4	4.15	3.43	1.29	0.47	0.69	0.80	0.33	0.9	10	341, 367, 394, 395, 414, 456, 471, 501, 779, 784 **(10)**
UB	10	4.65	3.14	1.24	0.53	0.64	0.68	0.17	0.3	5	341, 394, 463, 471, 478 **(5)**
UC	13.0	4.35	2.96	1.17	0.55	0.62	0.65	0.14	0.1	2	395 448 **(2)**
UE	18	5.35	3.26	1.32	0.59	0.67	0.69	0.12	0.1	1	471 **(1)**
UH	4.7	3.35	2.74	1.03	0.55	0.58	0.65	0.10	0.1	1	779 **(1)**
UN	20	5.55	3.49	1.35	0.59	0.67	0.69	0.12	0.3	6	353, 395, 463, 448, 478, 753**(6)**,
UP	7	4.70	3.44	1.32	0.66	0.68	0.73	0.03	nd		nd
Grand av.	**11**	**4.59**	**3.21**	**1.24**	**0.56**	**0.65**	**0.70**	**0.14**			

Key: N=Number of genotypes; Na = Number of different alleles; Ne = Number of effective alleles; I = Shannon Information/diversity Index; Pa= Rare/private alleles (alleles unique to a single population); Ho=observed heterozygosity; He = expected heterozygosity; UHe = unbiased expected heterozygosity; F=Fixation index; nd=not dtected

3.4 Discussion

Table 3.3 shows the 32 SSR markers that evaluated the genetic diversity of cultivated *Coffea canephora* in this work were efficient and reliable in detecting polymorphism as shown by different marker accession variability (Table 3.4, 3.6, 3.9). The low quality index values and average minimum variance coupled with ability to identify allele numbers, frequency, ranges, abundance and rare ones to the level of differentiation implied the markers further emphasized the polymorphic nature of enzymes. High allele availability range of 0.73 (marker DL011) to 1.0 (marker 471) with a mean value of 0.94 implied detected alleles were common among accessions. Major allele frequency range of 0.11 (marker SSR 755) to 0.86 (marker DL011) and a mean score of 0.37 justified the usefulness of all the 32 markers for identifying genotypes for crop improvement and conservation. The high genetic diversity range of 0.26 (marker DL011) to 0.94 (marker 755) with an average of 0.75 coupled with a high genotype number of 11 (SSR DL026) to 116 (SSR 755) with a mean value of 40.88 offer a wide variability range for selection. Even after selecting 18 markers with less than 50% missing data, genetic variation measures did not change a great deal (Table 3.7).

PIC scores of less than 0.50 realized only in markers DL011 (0.26) and 384 (0.43) (Table 3.4) while the 30 markers had values of up to 0.94 (Tables 3.4; 3.7) implicated high numer of alleles with high frequency which is desirable for crop improvement. Even SSR DL011 and SSR 384 with the lowest PIC values of 0.26 and 0.43 had allele frequencies of 20% and 65% respectively; implying can still be useful for breeding purposes even if the PIC values were relatively low. Genetic diversity measure relationships were positive and at times significantly (Table 3.5; 3.8), implying that the effects of genetic measures are reflected on others directly. Because the 32 markers were able to detect accession genetic variability, they can be utilized for future genetic evaluation of coffee particularly *C. canephora*. Most of the accessions were dominantly farmer landraces and could have evolved naturally through

reproductive isolation or differentiated by intra-specific divergence of a small part of the domesticated wild gene pool (Van Hintum., 1994).

Except for markers SSR DL011 and DL013 (Table 3.4), the rest of the 30 markers able to detect rare alleles in numbers that ranged from 4 (SSR 782 and DL026) to 24 (442) (Tables 3.4; 3.6), reflecting marker thoroughness in investigating DNA. Selection among other pressures could have contributed to absence of rare alleles in Jinja, Kayunga and Kituza progenitor germplasm (Table 3.14). Rare alleles were generally more in farmer Robusta coffee fields dominated by land races and in controls from West African than in germplasm collections (Table 3.14). Rare alleles are desirable for conservation while abundant alleles are suitable for research. Field gene banks in various research institutions, however, represent a tiny fraction of the immense genetic diversity available in farmer landraces and wild forms. Crop evolution, farmer complex selection pressures and highly variable biophysical adaptation systems are depressed in field gene banks. Coupled with research high selection pressure, a few narrow genetic base improved varieties as compared to farmer complex selection pressures that accommodate variable cultivars are developed. Different and effective alleles were generally higher in many locations particularly in Kamwenge and Kyenjojo at the boundaries of Kibale forests as compared to the germplasm collection (Table 3.14). The Kawanda, Entebbe botanical gardens as well as Kituza Robusta collections had continued loss of some trees to old age, pest and diseases without replacement. It is also likely that loss of original mother plant distinct traits and some genetic variability could have contributed to the failed consolidation of genotypes from the germplasm collections into distinct groups (Figure 3.7a; 3.7b). Whereas open pollinations that the germplasm collections were earlier subjected to could have contributed to the high observed, expected and unbiased expected heterozygosity among the germplasm collections (Table 3.14).

The Robusta coffee germplasm collections had also low allele frequency than their cultivated counterparts (Table 3.14). Markers with fewer genotypes and high allele frequencies are useful for discrimination and are ideal for selection, particularly so in the event of limited resources. The shorter the distance between desirable alleles, the better for breeding purposes because linked alleles have higher chances of being represented together in the hybrid progeny (Peakall et al., 2006). When alleles are far apart, they are likely to be separated during crossover of chromosomes (Brown et al., 1995). In this study it was found that all the two sets of research improved varieties coded UC and UH in Figures 3.3; 3.4 though belonging to different genetic diversity groups had a narrow genetic base as each lot was mainly distributed in the same genetic diversity group. In advent of calamities such disease out breaks as was the case of coffee leaf rust (Wrigley, 1988) and coffee wilt disease (Adipala et al., 2002), genetically distant genotypes stand chance of completely not being wiped out as some varieties will resistant attack while others succumb.

Commercializing genotypes with a narrow genetic base also limits their recommendation in multi locations because genotype suitability to specific niches is influenced by inherent traits (Leroy et al., 2011). As indicated in Tables 3.4-3.14 and Figures 3.1-3.5, Uganda is endowed with a wide range of *C. canephora* variability and therefore the variable gene pool can be utilized in crop improvement. Crossing genetically distant genotypes improves function, reduces inbreeding depression in populations and improves fitness in form of improved seed set and quality, germination, seedling survival, and resistance to stress and other production constraints. In Table 3.10, cross pollinating plants from different locations due to high variability within populations (82.97%) and within individuals (54.05%) potentially offers highly segregating population for selection of desirable agronomic traits.

Population genetic variability values of 54.05% within accessions, 28.92% among accessions within populations and 17.03% among populations (Table 3.10) were similar in trend to those found by Musoli et al., (2009) on wild Robusta coffee; 66.7% within individuals 19.9% among individuals within groups and 13.5% among populations. The genetic genetic differences among populations and individuals were still of *C. canephora* species. Quantitative variations for instance coffee wilt disease incidence as found by Musoli et al., 2013 pointed out involvement of many genes variably distributed among genotypes with the most resistant presumably having the largest number of resistance genes. AMOVA findings indicate that the contribution of geographical distribution to genetic variability was the lowest as compared to individual genotypes and genotypes within populations. The 17.03% variation among cultivated populations may have resulted from the wide distribution of Ugandan *C. canephora* with strong adaptations to rainfall and soil types (Thomas, 1935). The 82.97% genetic variation within populations may have partially been influenced by widespread distribution of Ugandan *C. canephora* with strong adaptations to rainfall and soil types. Robusta coffee grows naturally between 1000 m.a.s.l (Zoka forest) and 2000 m above sea level (Kibale forest) and in other forests located in Bunyoro, Buganda and in the North close to the Sudan boarder (Thomas, 1935, 1947; Leakey, 1970). Therefore significant genetic differentiation among Robusta coffee from locations or populations (Tables 3.10; 3.11; 3.12; 3.13) could have resulted from intra-specific divergence of a small part of the domesticated wild gene pool through genetic drift.

The results here estimated inbreeding index (F_{IS}) value of 0.35 that points out that Robusta coffee was dominantly out crossing (Table 3.11). Results also point out clearly that the distinctive characteristics of “nganda” and “erecta” wild types could have lost their originality through intra crosses, making it difficult to differentiate the two types clearly at genetic level, Figure 3.7a. Thomas, 1940 found 15%-40% of Ugandan Robusta types were

self fertilizing with 'nganda' types averaging 28% possibly causing many fruits to have only one developed bean. The inbreeding index value range of 0.05 to 0.98 for total samples (F_{IT}) with an average F_{IT} value of 0.55±0.06 pointed out that crossing among geographically distant genotypes was moderate. Population differentiation index (F_{ST}) average value of 0.31±0.03 confirmed a fair number of new alleles were introduced or exchanged among populations. Whereas SSR gene flow (Nm) average value of 0.7±0.09 verified one migrant was exchanged between populations in each generation. The high Shannon Diversity Index (H) values found among accessions from Kamwenge (1.71) and Kyenjojo (1.55) hinted not only to high genetic diversity but also to reduced habitat disturbance. The low H values found among "nganda" (0.35), Ugandan wild (0.48), Guinean controls (0.93) and Mukono (0.93) area may be associated with more disturbances in the habitat. Coffee pollen may contribute minimally to the distribution of alleles as is not sticky, light and carried by insects, wind up to a height of 8 m and a distance of 100 m (Leakey, 1970). Unless under special storage methods, where pollen can stay for over a month, the pollen losses ability to germinate after a few days (Wrigley, 1988). Wild animal and bird dispersal might also be limited to nearby locations. The only visible mode of long distance dispersal was by human movement of planting material from cuttings, seeds or volunteer seedlings from wild populations and farmer fields from one location to the other.

Three diversity groups that differed from the controls from West and Central Africa were formed in both UPGMA and PCA analyses verifying the reliability and preciseness of the two methods (Figures 3.4; 3.5; 3.6). One group was dominated by genotypes from Kabarole, Bundibudyo in the Western highlands and Hoima in the Lake Albert Crescent region. A second group comprised genotypes from adjacent districts of Kyenjojo in the Western highlands, Kiboga in the Lake Victoria Crescent bordering Lake Albert Crescent region and Kamuli at the far South East. The third group constituted accessions from Kayunga, Mpigi,

Mubende, Rakai, Mukono from Lake Victoria Crescent and Kamwenge from Western highlands. The widely distributed genotypes of different diversity groups make *in situ* conservation strategies expensive.

Whereas cultivated Robusta coffee in this study revealed 3 genetic diversity groups, Musoli et al., (2009) had classified them into four that comprised cultivated (nganda and erecta), feral from Kalangala, Itwara and Kibale forests. According to Curbry et al., 2013, Ugandan wild *C. canephora* genetic diversity group and Nemaya genotypes introduced in South America early in the 19 century belong to the Congolese SG2 subgroup in Central Africa. Besides SG2, other Congolese subgroups include B from the Congo Basin, C from the Central African Republic and Cameroon, and SG1 from the Atlantic coastal region of central Africa near Gabon (Leroy et al., 2006). The Congolese SG2 and SG1 subgroups constitute two *C. canephora* genetic diversity subgroups grown worldwide (Curbry et al., 2013; Thomas 1935). Whereas the origin and enormous genetic diversity of the main Congolese and Guinean groups has attributed to the last glacial maximum (LGM) refuges and paleoclimatic events 18,000 years BP (Berthaud 1986; Anthony et al., 2010).

The distint differences observed between the three genetic diversity groups of cultivated Ugandan *C. canephora* and those from Western and Centarl Africa may have resulted from use of few controls. The genetic and phenotypic diversity groups derived from the study confirm earlier observations that Uganda is within the corridor of *C. canepora* (Maitland, 1926; Thomas, 1940). The locational constitution of the three derived diversity groups point out an important social aspect of farmers sharing or acquiring planting material from other districts or regions in a random fashion. The Robusta seed distribution scheme of 1923/4 that aimed at increasing Robusta coffee acreage and small scale farmer tendencies of sourcing free planting material from forest and fellow farmers (Maitland, 1926; Thomas, 1935) could

have contributed to the placement of accessions from far and nearby locations in similar diversity groups.

CHAPTER FOUR: MORPHOLOGICAL AND BEAN PHYSICAL DIVERSITY OF CULTIVATED *Coffea canephora* IN UGANDA

4.1 Introduction

Coffee plant descriptors developed by International Plant Genetic Resources Institute (IPGRI, 1997) have and still can be used to evaluate and select highly productive cultivars (Thomas, 1940; Leakey, 1970; Wrigley, 1988; Kibirige-Sebunya eta al., 1993). According to Aga et al., 2003, some heritable material is reflected by morphological descriptors despite the combined effects of genotype, growth stages and environmental. In soybean and durum wheat, wild genotypes were classified into groups and their differences determined (Dussert et al., 2004) using agro-morphological marker data.

The Robusta coffee bean like most other food products possess characteristics which are related to their state, aspect or appearance such as weight, volume, size, shape, colour, solubility, moisture content and texture. The characteristic quality expressed by coffee green bean reflects inherent traits and growth surrounding conditions (Leroy et al., 2006). The size, shape of Robusta beans, color, chemical composition and flavor to a great extent were genetically influenced Charrier et al., 1985. In addition, the different environments and management farm practices might have significant contribution to physical characters and biochemical composition (Lu et al., 1997; Davrieux et al., 2003). For instance coffee beans from high altitude are more greenish in colour, small, denser, harder and more appreciated due to higher acidity, good aroma and flavour than that from lower altitudes (Lerroy et al., 2006). Different markets also prefer different physical bean characters provided they have acceptable visual aspect, are homogeneous and their production is regular and reliable. Bean size grade from a commercial point of view is an important factor since price is related to the coffee grade (Leroy et al., 2006). Moisture content of 12.5% and above is regarded as a fault

for a trader or a roaster as it results to greater roast weight loss. Various bean physical characters determine the way coffee is processed.

Understanding farm phenotypic diversity is a pre requisite in enhancing Robusta coffee landraces utilization in crop improvement program, Farm management practices differ with respect to shade trees, soil fertility, weed, canopy, disease and pest control practices that modify plant growth, development and bean quality. Furthermore genotypes respond differently in different environments. Knowledge of morphological and bean physical character diversity of cultivated Robusta coffee would simplify decision making on what germplasm and natural habitats to conserve to mitigate the effects of genetic erosion. In addition, traditional breeding relies on morphological characters when choosing parental materials for breeding program, the requirement for the exploitation of useful traits in plant breeding (Van der Vossen, 1985). Even with modern assisted marker breeding, successful generation of variability outside natural processes requires species' basic information on existing variability on both phenotypic and genetic variability (Montagnon et al., 1996; Leroy et al., 1993).

However, there is limited information on farm Robusta coffee tree and bean morphological diversity. The wide range of phenotypic variation observed among cultivated Ugandan Robusta coffee (Maitland, 1926; Thomas, 1940) did not sufficiently provide statistical evidence. The existing germplasm gene pool could have been narrowed by the selection criterion that emphasized high productivity (Leakey, 1970) leaving out other important agronomic aspects such as quality and drought tolerance. It is therefore probable that the few representatives of domesticated and wild seedling progeny at Kawanda field gene bank represent a small fraction of original parent stock or immense genetic diversity that could be in farmer fields and the wild. This chapter was therefore designed to characterize the

variability of coffee tree morphology and green bean physical parameters spread over a wide diverse geographical area for purposes of conservation, crop improvement and marketing.

4.2 Materials and Methods

4.2.1 Site and tree identification

Stratified purpose sampling of non overlapping geographical areas was adopted. Six traditional Robusta growing regions of West, Central, East and South West represented by six broad agro ecological zones, namely, Lake Victoria crescent, Lake Albert crescent, South East, Southern highlands, Southern dry lands and Southern highlands were sampled (Figure 4.2). Accessions were collected from 21 districts and three gene bank collections of Kawanda, Kituza and Entebbe botanical gardens (Table 4.1). To ensure diversity is captured during sampling, a distance of 5 kilometers between a group of 4 farmers was considered in each district. For districts with forests such as Kabarole and Kamwenge, farms close to the wild were included. In all cases, trees older than 10 years and above were chosen for sampling on assumption that they were well adapted and chances of being land races was high. A recommended sample size of 25-30 trees and above per population was considered adequate to address questions related to genetic distance (Meng et al., 1994). At Kawanda where earlier seed germplasm collection missions were planted in 1935, field records were used to select the 59 trees based on coffee types; “nganda”, “erecta” or hybrid. Entebbe botanical garden, planted before 1902 (Wrigley, 1988) had 10 trees selected while all the 16 controlled Robusta crosses planted at Kituza were evaluated.

4.2.2 Morphological data collection

Passport data consisted of the following information; farmer name, village, parish, sub county, district, field agronomic management practices, use of compost manure, inorganic manure, chemical or organic sprays for infestations, pest and disease occurrence, intercrop any and shade trees. Individual farmer discussions using a check list was used to enter

information such as acreage, source of planting material, tree age. The 15 qualitative and the 5 quantitative descriptors are as shown in (Table 4.2.1; 4.2.2). Masindi district known for extensive cultivation of crops such as sugarcane and tobacco had only 12 coffee samples evaluated. The rest of the 20 districts had tree numbers that ranged between 15 and 26. In total 476 phenotypes had their morphology evaluated. In cases where the farmer was not certain about the tree age, it was estimated using the trunk of the tree in comparison to the standard. The nature of infection was recorded. Randomly selected ripe cherry clusters not earlier picked were counted and size estimated using of small, medium or large size. Inter node length was measured between two nodes of a primary in centimeters (cm). Leaf length was measured from leaf petiole along the mid rib to the apex in cm. Leaf width was measured about half leaf length in cm. Each of the Robusta coffee tree attribute was measured at least 10 times and an average was calculated. Tree production capacity and vigour was graded on a 1-5 scale (where 1= very few berries present in the tree; 5 = Very many berries in the tree and 1= unhealthy tree growth; 5 = healthy tree growth respectively). The location of the study site was taken using Geographical Position Systems (GPS). Trees were characterized as “nganda” or “erecta” types using growth habits like drooping nature, upright, colour and size of leaf. All morphological characterized trees were tagged for future reference. Qualitative characters were scored as detailed in Table 4.2.2.

Table 4.1: International Plant Genetic Resources Institute coffee descriptors used to characterize the morphology of cultivated Robusta coffee

Table 4.1.1: Qualitative characters

Character description	Descriptor states
1. Tree shape	conical, intermediate, cylindrical
2. Tree growth habit	open, normal, compact
3. Tree height	tall, medium, small
4. Angle of primaries	semi erect, nearly horizontal, horizontal
5. Primary secondary branching	profuse, normal, slight
6. Strength of primaries	thin-droop, average, thick strong
8. Colour of flush leaves	bronze, light bronze, green
9. Colour adult leaves	dark green, green, light green
10. Shape of leaves	ovate, round
11. Surface of leaves	undulated, intermediate, flat
12. Size of fruits	large, medium, small
13. Shape of fruits	pointed, elliptic, small
14. Fruit disc size	large, medium, small
15. Disease reaction	present, absent

Table 4.1.2: Quantitative characters

Nature	Character description	Measure
Tree	Number of stems in a tree	Counts
	Tree age	Centimeters
	Tree girth	Centimeters
	Production capacity	Rating
	Vigour	Rating
Primaries	Primary internode length	Centimeters
Leaves	Leaf length	Centimeters
	Leaf width	Centimeters
Fruits	Fruit number per axil	Counts

Rating of some quantitative characters

PC: 1 = very few berries present in the tree; 5 = Very many berries in the tree

Vigour: 1 = Miserable/unhealthy tree growth; 5 = Healthy tree growth

4.2.3 Harvesting and drying Robusta coffee ripe cherry

All the 476 tagged farm Robusta coffee trees, whose phenotypic characteristics were evaluated had least 2 kilograms ripe cherry harvested from each tree as shown in Table 4.1. Most locations in the Northern hemisphere had their ripe cherry collected during the main harvest of second season 2008 (September to January 2008) while the Southern hemisphere was considered during main harvest of first season 2009 (April to June 2009) (map 6.1). Harvested ripe cherry was poured in a bucket of water and beans and debris that floated was

removed before sun drying in wire mesh boxes. Other coffee defects were removed from the drying trays. The ripe cherry, with estimated initial 50% moisture content (Clifford et al., 1985) was spread to a maximum of 1.5 cm layer thickness to avoid formation of mould or bean deterioration. The coffee was regularly turned until it achieved 12.5% moisture content. Drying lasted for about a month and the dry coffee bean was stored in a dry aerated room.

The dried cherry referred to as ("Kiboko") was carefully hulled. A metal tray was used to winnow to separate physically defective cherries and bean hull from clean beans based on density. Beans with defects were sorted from normal green bean. Beans with defected beans such as molted beans can produce unpleasant and unpalatable coffee flavour. Clean green beans were stored in polythene bags and placed in an aerated dry place awaiting evaluation of green bean physical characters, biochemical compounds, nutrient elements as well as organoleptic cup test. In all the drying and storage process, caution was taken not to expose drying cherry, dried Kiboko and coffee beans to water or humid environments.

4.2.4 Evaluating green bean physical characteristics

A representative sample size of 206 Robusta coffee genotypes was selected based on Near Infra Red Spectroscopy fingerprint and data on major biochemical compounds that influence cup quality as indicated in Chapter 6. The 206 samples comprised farm collections from 21 districts, Kawanda and Kituza Robusta germplasm collections. Beans of three other samples with proven cup test characteristics by Uganda Coffee Development Authority (UCDA) were included as controls. Because of different farm management practices and tree selection criteria that emphasized diversity rather than tree yields, some genotypes had less than 100 grams. To minimize bias, samples were numbered and randomly picked for evaluation. 300 grams of green beans were measured out. Green beans were graded according to their bean size using different and specified screen size holes of that varied from 20 (8 mm) to 8 (3 mm)

diameter. Screen sizes used for the study included large (A) ≥18 (7.0 mm), medium (B) ≥15-<17 (6.0–< 6.75 mm) small size 15 (6.0 mm), and empty beans (PB) ≤12 (≤ 4.5 mm). The coffee beans that remained in each screen were weighed and the percentage recorded. Average weight of 100 seeds was taken four times and overall mean was obtained in grams. Clean green beans colour, shape, smell, pea beans, hollow beans, fadedness (paleness), varying thickness of silver skin, general appearance, nature of defective beans, bean homogeneity, roasted bean centre cut was noted. Bean colour provided information about the freshness, moisture content, homogeneity, good and poor processing. Analytical scale was used to weigh green and roasted green beans in grams. Green and roasted bean volume was measured using a volumetric cylinder in cubic milliliters and their difference provided roast bean volume increase. Roast time per gram (RTPG) was calculated in seconds by dividing total roast time with total weight of green beans roasted. Percentage weight decrease of roasted beans measured as green bean percentage weight loss was acquired by expressing the difference in green and roasted beans as a percentage. The percentage increase in volume of roasted beans was obtained by expressing the difference in green and roasted bean volume as a percentage.

4.2.5 Data analysis

Microsoft Excel 2007 pivot table was used to convert qualitative phenotypic data frequencies into percentages to calculate minimum, maximum, range, mean and standard error values. Shapiro-Wilk test in XLSTAT version 2011.2.05 statistical program (Addinsoft, Paris, France) was used to establish a non parametric distribution of data. Spearman correlations were used to quantify the strength of the relationship between a pair of morphological characters measured on ordinal scale (interval scale can also be used) at 5% significance level. Stronger relationships were reflected with larger values. Altitude and tree age for optimum traits expression was estimated using Gaussian species packing analysis imbedded

in Palaeontological Statistics Program 2.14 (PAST). PAST program was used because of its ability to handle more than one dependent variable without significant influence of independent variable correlations.

Effect of tree age and altitude on morphological traits was obtained by multiple regressions between coffee tree age, altitude (independent) and 476 individual morphological parameters (dependent) was analyzed using the regression equation; $y = a + bx$ (a =constant or y axis intercept; b = regression coefficient or slope; x= interdependent variable, y= dependent variable) in GenStat 12th edition.

Comparison and significant differences between means of one or more factors or categories (coffee types, soil texture, tree age and altitude ranges) was established using ANOVA.

To compare factor means and identify factors that impose significant differences to morphological attributes, XLSTAT version 2011.2.05 statistical program was used for Analysis of Variance (ANOVA) of data. The factors included, soil texture (clay loam, sandy loam, loam), Robusta coffee types ("nganda", "erecta", commercials, hybrids), tree age range (4-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80 years), site altitude range (697-1000, 1001-1100, 1101-1200, 1201-1300, 1301-1400, 1401-1500, 1501-1654 meters above sea level) and 23 locations. Because the sampled population was assumed not normally distributed, t-test was used to identify any significant differences between means. The coefficient of determination (R^2) in correlation, regression and ANOVA analyses was used to measure the proportion of variability in one variable that was accounted for by another. The unaccounted variation was attributed to other factors outside the two assessed variables. To separate the means beyond ANOVA, multiple ranges Tukey Honestly Significant Difference (HSD) analysis of variance at 95% confidence was calculated. Bar and line graphs were then

drawn using Microsoft Office Excel 2007 to compare mean phenotypic character values under different factors.

Bartlett's specificity test in XLSTAT version 2011.2.05 statistical program was carried out to establish significant differences from zero among correlated phenotypic parameters before performing Principal Component Analysis (PCA). The PCA was needed to aggregate genotypes with similar morphological characteristics into high internal homogeneity and high external heterogeneity as in chapter 3, section 3.6. In this chapter, pair wise comparison of morphological parameters was done to derive a multi dimensional scatter plot of individuals. Genetic distances for phenotypic characteristics were estimated using Euclidean straight line method (Mohammadi et al., 2003). Correlation matrix standardizes original data parameters measured with different units such as morphology, bean physical characters. Varimax rotation in XLSTAT version 2011.2.05 statistical program was used to improve the principal component plot reliability (Mohammadi et al., 2003). Genotypes were then assigned to principal components that had the highest attribute variance.

A non hierarchical clustering K means analysis procedure categorized genotypes that were closely related beyond PCA analysis (Mohammadi et al., 2003). Three K means groups for bean physical characters were adopted after ascertaining that not all the four genetic diversity groups portrayed by the PCA were different populations using Mahalonobis distance values. Group means in K clustering were created based on un-weighted paired group mean algorithms. A similarity index between phenotypic characters of different genotypes calculated the distance of each phenotypic character from average and determined whether the accessions were from the same or different populations. The number of K means analysis groups were pre-determined with the PCA distribution plot and Multi Dimensional Scaling (MDS) of genotype and parameter geometric distance method (Mohammadi et al., 2003).

MDS is capable of reflecting differences between many accessions and close individuals and does not assume linear pattern of variance. Defining many groups using K means analysis minimized differences between groups. Descriptive statistics was used to characterize different groups of phenotypic character using mean, median, variance, inter quartile range in form of box plots. Subsequently, the factorial step discriminant analyses spatially distributed the K means analysis derived groups on principal components. Mahalanobis and Fisher inter-group distances at 95% probability were also calculated using factorial step discriminant analyses. Once this was done, the confusion matrix estimated the efficiency of genotype placement among groups in percentages.

Percentage similarity variance contributed by each of the phenotypic attributes to group formation and mean group abundance was estimated using Bray-Curtis distance measure for both Robusta coffee types and K means analysis. One way multivariate analysis of similarity (ANOSIM) with 9999 permutations further verified whether there were significant differences in K means analysis group mean rank within and between variance at 95% probability (derived groups) (Mohammadi et al., 2003).

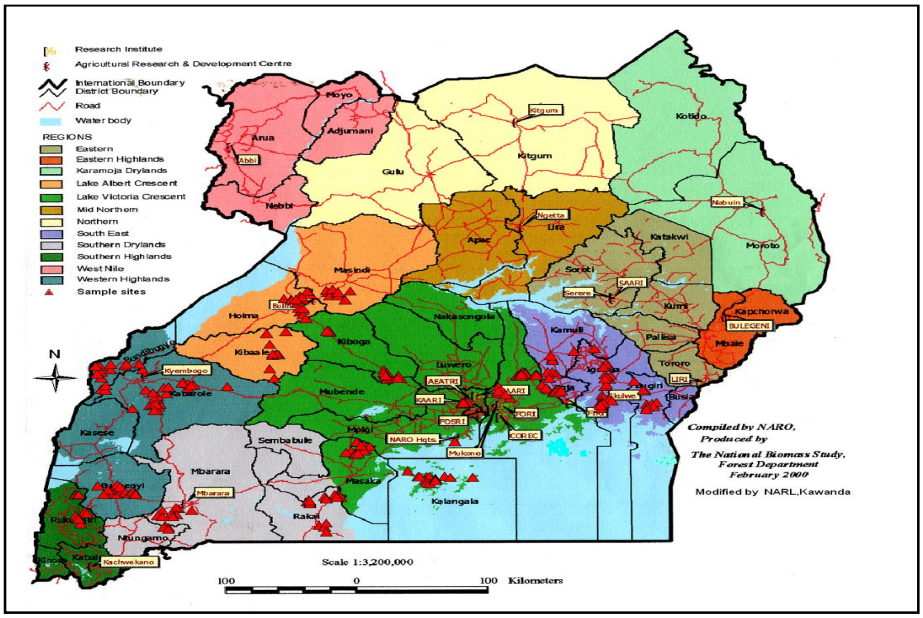


Figure 1: Ugandan locations and agro ecological zones where farm Robusta accessions were collected

Table 4.2: Cultivated *C. canephora* samples collected for phenotypic evaluation with geographic and climatic information from 23 sites in Uganda

Region	Agro ecology	District	Code	Phen Eval	Samples collected					Range					
					Year of NIRs bio compound analysis			Phy & org	Elmt SLB	Latitude (^{o}N/S)		Longitude (^{o}E)		Altitude range (m)	
					2004	2005	2010			Min	Max	Min	Max	Min	Max
East	South East	Mayuge	MY	18			18	3	4	N0042931	N0051757	E03340857	E03349122	1181	1217
		Bugiri	BG	17			17	6	3	N0042224	N0092647	E03306035	E03313644	1080	1120
		Jinja	JJ	18	5	3	18	10	4	E0055273	N0056411	E03306366	E03314627	1124	1184
		Mubende	MB			11									
		Kamuli	KM	22	15	11	22	13	3	N0042224	N0092647	E03306035	E03313644	1080	1120
		Iganga	IG	17			17	4	2						
Central	Lake Victoria crescent	Mukono	MK	15	7		15	10	3	N0048242	N0055842	E00271119	E03282498	1122	1176
		Kayunga	KY	20	8	9	20	8	4	N0064381	N0075396	E03287969	E03294904	1088	1105
		Kiboga	KG	21	6		21	10	4	N0067717	N0070590	E03198299	E03206659	1136	1373
		Masaka	MA	18			15	12	3	S0001152	S0048381	E03153688	E03183681	1202	1283
		Kalangala	KL	18			18	9	2	S0017794	S0020928	E03208763	E3210964	1028	1239
West	Lake Albert crescent	Masindi	MS	12			12	4	2	N0159805	N0160711	E03164147	E03168038	1152	1180
		Hoima	HM	26	10	24	26	12	2	N0132166	N0152893	E03134600	E03143832	1101	1295
		Kibaale	KI	21	13	19	21	5	4	N0077980	N0116687	E03107470	E03133531	1110	1247
	Western highlands	Kyenjojo	KJ	23	7	10	23	9	2	N0060199	N0065620	E03046422	E03064942	1372	1517
		Kabarole	KB	18	5	6	18	7	4	N0037968	N0074184	E0302950	E03037691	1009	1568
		Kamwenge	KW	26	8	23	26	12	4	N0040884	N0042520	E03039998	E03048180	1242	1325
		Bundibidyo	BU	16			16	10	3	N0067017	N0081142	E0300453	E03015030	687	1017
		Bushenyi	BS	19			19	8	3	S0022584	S0029433	E03007170	E0308698	1015	1654
South West	Southern drylands	Rakai	RK	18	3	2	18	13	3	S0050293	S0083097	E03152981	E03163619	1212	1230
		Mbarara	MR	19			19	7	1	S0054500	S0085342	E03038264	E03070577	1383	1478
	S. highland	Rukungiri	RG	18			18	9	4	S0035639	S0050771	E02943645	E03011327	1015	1559
	On station germplasm	Kawanda	KA	50	27	17	59	16	2	N0041700	E03253300	N0041700	E03253300	1177	1177
		Entebbe	EB	10	3		10		1	S0033333	E03245000	S0033333	E03245000	1177	1177
		Kituza	KZ	16			17	8		N0045000	E03275000	N0045000	E03275000	1200	1200
		Controls	CN					3							
				476	114	135	487	208	67						

Key to Table 4.2: Phen eval=No of Accessions evaluated on site; NIRs=No of smaples evalutead for...Near Infra Red spectroscopy; Phy & org=No of Samples evaluated for bean physical and organoleptic cup; Elmt SLB=No of Collections evaluated for soil texture as well as soil, leaf and green bean nutrient elements.

4.3 Results

The percentage occurance of cultivated Robusta coffee types, growth habit leaf colours, shapes and surfaces is shown in Figure 4.2a, b. The “nganda” (48%) and “erecta” (40%) were the dominant cultivated coffee types (Figure 4.2a). Commercial types constituted 9% and hybrids 3% of cultivated Robusta coffee. Over 75% of coffee trees had cylindrical shape, 20% were intermediate while less than 5% had a conical shape. Dwarf trees in farmer fields constituted 1%. Normal and open growth habit were almost at equal rating totaling to 99%. About 78% of trees were tall while another 20% had normal tree growth. Trees with average strength were 50% while 47% were of thick and strong nature. Thin and weakly bending trees constituted 3% of study samples. Young leaves were dominantly green (65%) with some light bronze shades (20%) and bronze (15%) (Figure 4.2b). About 80% of adult leaf colour was green, 20% were either light or dark green. Adult leaf surface was dominated by intermediate undulated type (70%). Flat surfaced leaves occurred at about 25% whereas undulated leaf had 5%.

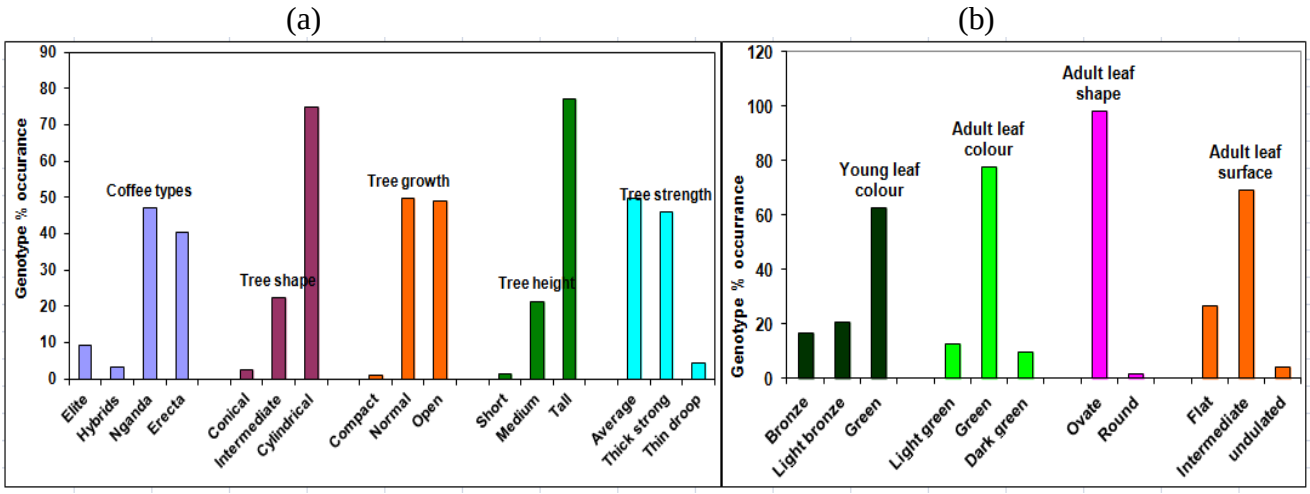


Figure 4.2: Occurrence of cultivated Robusta coffee (a) types and growth habit (b) leaf colours, shapes and surfaces

In Table 4.3, it is clear that, the “erecta” and “nganda” types had older trees of 37 years mean age compared to the commercial and hybrid types that had a mean age of 12. The lowest mean production capacity was from “nganda” and “erecta” types (3.30) while the hybrids had an intermediate value of 3.56 and the commercial types 3.73. The hybrids were as vigorous as the commercial types while the “nganda” and “erecta” had similar vigour (3.2). The “nganda” types had the highest mean stem number (6.63) followed by “erecta” (5.09) while the hybrids had the least (3.25). Commercial types had the longest mean inter node length (6.39) followed by “erecta” (6.21), “nganda” (5.98) and least by hybrids (5.09). As for the leaf length pattern, the hybrids had the longest mean leaves (19.94cm), followed by the commercial types (19.17units) and least by “nganda” (17.89uints). Regarding mean leaf width, the hybrids had the narrowest leaves (6.96 units) next by the “nganda” (7.36 units), while “erecta” types had the broadest leaves (7.47uints). The commercial types had the most fruits (21.93) while hybrids had the least number (16.49). Value ranges were highest in the “nganda” and “erecta” types hinting existence of possible high variability. Overall morphological parameter mean values were similar to those of “nganda” and “erecta”, reflecting the dominating influence of the two coffee types.

Table 4.3: Morphological diversity of 476 cultivated Robusta coffee types

Types	Statistic	Age	Girth	PC	Vigour	Stems	IL	LL	LW	Fruits
Com No. 44	Range	30	68	3	2	10	4.7	14.3	5.2	18.3
	Mean	11.68	46.55	3.73	3.55	4.09	6.39	19.17	7.38	20.93
	s.e.	1.41	2.72	0.10	0.09	0.30	0.19	0.40	0.16	0.56
Hybrids no. 16	Range	0	25	2	2	3	1.6	7.8	3	10.1
	Mean	12	47	3.56	3.56	3.25	5.09	19.94	6.96	16.49
	s.e.	0	2.90	0.16	0.16	0.21	0.12	0.43	0.21	0.60
Erecta no. 191	Range	76	159	4	4	21	6.8	21.2	6.8	27.8
	Mean	37.01	69.77	3.30	3.23	5.32	6.21	18.35	7.47	20.62
	s.e.	1.58	2.25	0.05	0.04	0.24	0.09	0.19	0.08	0.36
nganda no. 224	Range	75	205	4	4	27	5.9	13.7	9.5	31.4
	Mean	37.39	74.30	3.26	3.18	6.63	5.98	17.89	7.36	19.92
	s.e.	1.49	2.39	0.05	0.04	0.26	0.07	0.15	0.08	0.30
All types No.476	Range	76	205	4	4	27	6.8	21.2	3.1	31.4
	Mean	33.95	68.93	3.33	3.24	5.76	6.08	18.26	7.39	20.19
	s.e.	1.03	1.52	0.03	0.03	0.16	0.05	0.11	0.05	0.21

Key to Table 4.3: com=commercial types; s.e=standard error; PC=production capacity (scale 1-5); IL=inter node length (cm); LL=leaf length (cm) LW=leaf width (cm)

"Nganda" and "erecta" types were much older trees as compared to the commercial and hybrid types (Figure 4.3a). The hybrids had the longest leaves while the "nganda" had the shortest (Figure 4.3b). The commercial types had the most fruits whereas the hybrids had the least. The hybrids and commercial types were the most productive than "nganda" and "erecta" (Figure 4.3c). The "nganda" had the most stems while the hybrids had the least. Inter node length was much longer among the commercial types and shortest in the hybrids.

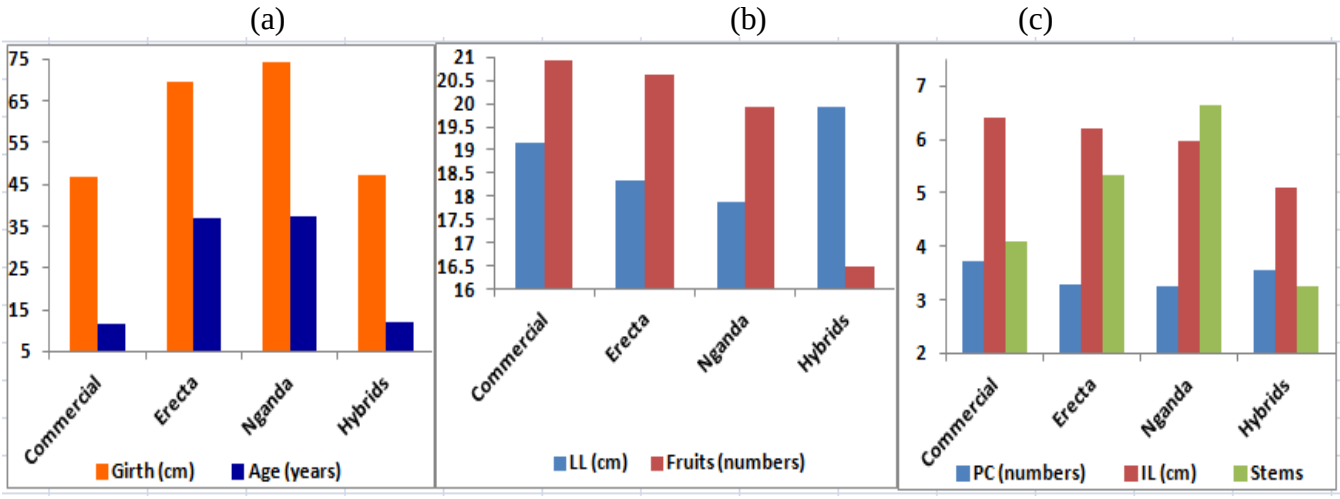


Figure 4.3: Comparison of Robusta coffee type morphological attributes of 476 genotypes

PC=production capacity (scale 1-5); IL=inter node length (cm); LL=leaf length (cm)

The longest Mahalanobis genetic distance was between the hybrids and the “nganda” while the shortest was between “erecta” and “nganda” (Table 4.4). The hybrids were different populations from “nganda” and “erecta” as they had Mahalanobis genetic distances of 4.40 and 4.06 respectively which were greater than 3. The Mahalanobis genetic distance value of 2.79 between the commercial types and hybrids almost categorized the two as different populations. The Mahalanobis distance between the “erecta” and “nganda” (0.20); commercial and “erecta” types (0.84) commercial and “nganda” (1.50) did not qualify them to be different populations. As is the case with Mahalanobis distance, the longest genetic distance using Fisher estimates was between the hybrids and “nganda”, while the shortest was between “erecta” and “nganda”. Fisher probability that any two groups were different from each was highly significant (Table 4.4).

Table 4.4: Mahalanobis and Fisher distances for cultivated Robusta coffee types

	Mahalanobis distance				Fisher distances				p-values for Fisher distances			
	com	erecta	hybrids	nganda	Com	erecta	hybrids	nganda	com	erecta	hybrids	nganda
com	0	0.84	2.79	1.50	0	4.98	5.40	9.09	1	<0.0001	<0.0001	<0.0001
erecta	0.84	0	4.06	0.20	4.98	0	9.89	3.33	<0.0001	1	<0.0001	0.003
hybrids	2.79	4.06	0	4.40	5.40	9.89	0	10.84	<0.0001	<0.0001	1	<0.0001
nganda	1.50	0.20	4.40	0	9.09	3.33	10.84	0	<0.0001	0.003	<0.0001	1

Key to Table 4.4: com = commercial

Long internodes, many fruits, long and broad leaves were positively correlated to each other (Figure 4.4a). Tree vigour was associated with production capacity. The most unrelated coffee type morphology was between the hybrids and landraces, particularly that of "nganda" (Figure 4.4b). The commercial types and hybrids were also not associated with each other (Figure 4.4b). The commercial types were more related to the "erecta" than with "nganda" types. The "erecta" and "nganda" types were the most similar to each other. The "nganda" types had the most stems, shorter internodes, longer leaf length and width (Figure 4.4a, b). The "nganda" and "erecta" types were older trees. The commercial types had longest internodes, longest broad leaves, many fruits and fewer stems. The hybrids were the younger, most vigorous and productive.

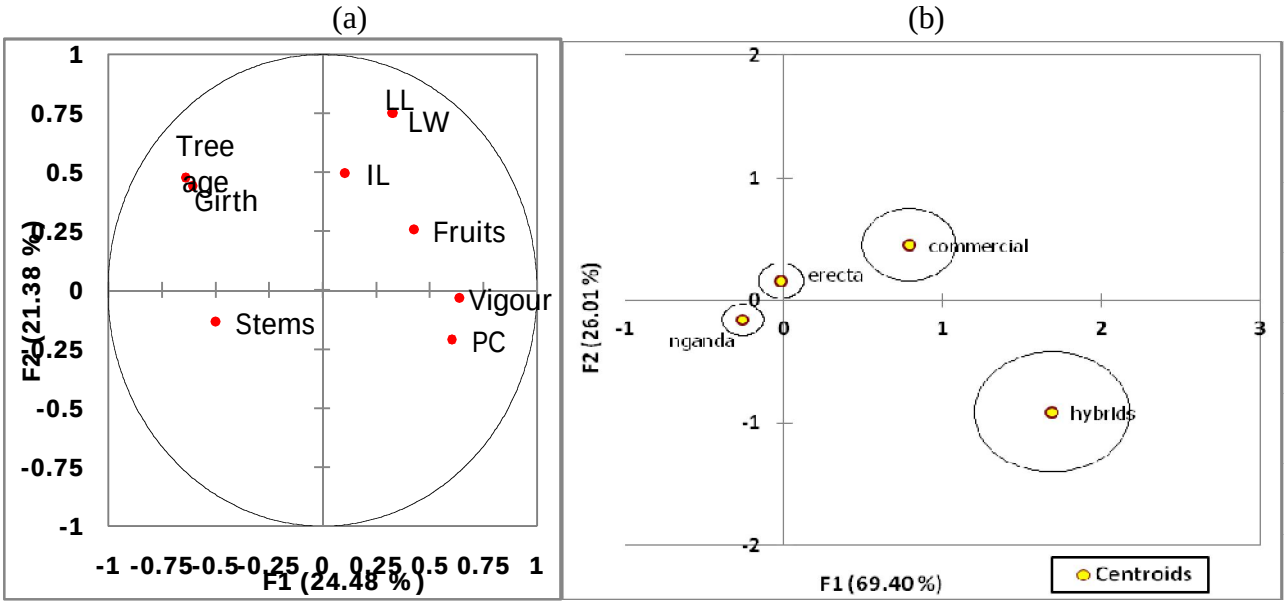


Figure 4.4: Relationships among a) morphological parameters b) Robusta coffee types

Key to Figure 4.4: PC=tree production capacity; IL=internode length; LL=leaf length; LW=leaf width

Phenotypic traits performed best at 1200 masl (Table 4.5). Optimum phenotypic trait performance was 32-35 years with a girth circumference of 19.42-64.94 cm.

Table 4.5: Estimated height and Robusta coffee tree age for optimum morphological parameter performance using Gaussian gradient.

	Age	Girth	PC	Vigour	Stems	IL	LL	LW	Fruits
Opt ALT	1188.6 ±134.9	1190.6 ±138.4	1200.2 ±146.7	1200.8 ±145.3	1192.4 ±142.1	1193.6 ±143.9	1196.1 ±141.3	1196.1 ±142.9	1197.0 ±143.9
Max	80	208	5	5	28	10.1	31.6	14	39.6
Opt tree age		42.18 ±22.76	32.86 ±22.26	33.29 ±22.37	36.41 ±20.64	34.23 ±22.65	34.13 ±22.71	33.97 ±22.52	33.95 ±22.48
Max		208	5	5	28	10.1	31.6	14	39.6

Key to Table 4.5: Opt=optimum; max=maximum; PC=tree production capacity; IL=internode length; LL=leaf length; LW=leaf width

Age range of 4-60 years significantly reduced tree girth (Table 4.6). Trees of 4-30 years were significantly productive while those of 4-20 years were significantly vigorous. Age range of 11-70 years had significantly many stems in trees. A significant reduction in internode length was realized from 4-80 year old trees. Trees of 41-70 years had significantly narrow leaves. Large leaves were apparent in 41-50 and 61-70 age range.

Table 4.6: ANOVA of phenotypic characters and tree age range of 476 genotypes

ANOVA statistic summary							
Mxtr	**R^2**	**v.r**	**p v**	**sdf**	**m.c**	**s.e**	**p v**
Girth	0.58	93.91	****	4-10, 11-20; 21-30, 31-40, 41-50, 51-60	-71.9, -59.4 -32.64, -49.1 -30.18,-9.2	3.7, 3.7, 4.0, 4.6, 4.2, 4.4	****,****, ****,**** ****, **
PC	0.06	4.17	****	4-10, 11-20, 21-30	0.49, 0.37, 0.30	0.12, 0.12, 0.12	****,***, **
Vigour	0.03	1.92	*	4-10, 11-20	0.29, 0.24	0.11, 0.11	***, **
Stems	0.20	16.58	****	11-20, 21-30, 31-40, 41-50, 51-60, 61-70	1.80, 3.16, 3.13, 4.52, 3.78, 5.29	0.56, 0.6, 0.68, 0.63, 0.65, 1.08	***,****, ****,****, ****, ****
IL	0.04	3.0	***	4-10, 11-20, 31-40, 41-50, 51-60, 61-70	-0.58, -0.68, -0.72, -0.79, -0.82, -0.84	0.20, 0.20, 0.24, 0.22, 0.23, 0.38	***, ***, ****,****, ****, **
LL	0.07	4.96	****	4-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70	-1.83, -1.25, -1.95, -1.88, -2.48, -1.85, -2.32	0.42, 0.43, 0.45, 0.52, 0.48, 0.49, 0.81	****, ***, ****,****, ****,****, ***
LW	0.03	1.76	*	41-50, 61-70	-0.64, -0.94	0.23, 0.38	***, **
F Stat; F7, 468							

*, **, ***, **** significant at 0.09, 0.05, 0.008, 0.0005 levels of probability

Key to Table 4.6: Mxtr=morphology character; R^2=coefficient of multiple determination; v.r=variance ratio; sdf=significantly different factor; m.c=model coefficient; s.e=standard error; p.v=probability value; PC=production capacity (scale 1-5); IL=inter node length (cm); LL=leaf length (cm) LW=leaf width (cm)

Robusta coffee tree girth increased with age (Figure 4.5a). Leaves of 11-20 year old coffee trees were slightly less longer than those of 61-70 years. Stems increased with age although trees of 61-70 had notably few stems. Trees of age range 61-70 had the longest inter node length as would be expected. Fast growing trees were found at elevation 1101-1200 (Figure 4.5b). Productive and vigorous trees were mainly located at 1301-1400 m above sea level. The least productive trees were found at height below 1101-1200 m above sea level while low tree vigour was located below 1200

m above sea level. The longest leaves were observed at elevation 1101-1200 m above sea level while the shortest mean record was at 1401-1500 m above sea level.

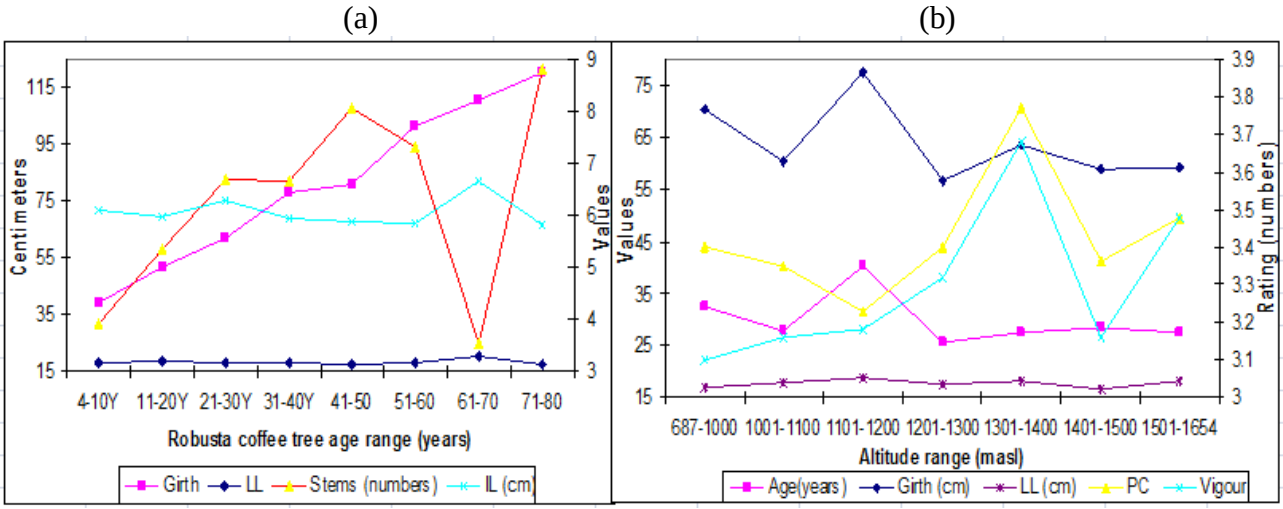


Figure 4.5: Line plot comparing morphological performance at different a) tree age ranges and b) elevation ranges

Table 4.7 compares phenotypic characters with altitude range. At altitude 1101-1200 masl, trees grew faster and acquired a larger tree girth. Trees at 1101-1200 m above sea level had significantly reduced tree vigour while those located at 1401-1500 m above sea level had significantly shorter internodes and leaves.

Table 4.7: ANOVA of phenotypic characters of 476 genotypes with altitude range

ANOVA statistic summary							
Mxtr	**R^2**	**v.r**	**p v**	**alt range**	**m.c**	**s.e**	**p v**
Age		0.09	7.40	****	1101-1200	12.58	4.91	**
Girth		0.08	6.50	****	1101-1200	18.12	7.23	**
PC		0.04	3.47	***				
Vigour		0.05	4.15	****	1001-1100	-0.31	0.16	**
Vigour		0.05	4.15	****	1101-1200	-0.30	0.14	**
IL		0.05	3.77	***	1401-1500	-0.77	0.34	**
LL		0.08	7.0	****	1401-1500	-1.46	0.72	**
LW		0.03	2.75	**				
F Stat; F6, 469								

*, **, ***, **** significant at 0.05, 0.0001 levels of probability; morphology character; R^2=coefficient of multiple determination; v.r=variance ratio; p.v=probability value; m.c=model coefficient; s.e=standard error; PC=tree production capacity; IL=internode length; LL=leaf length

Bushenyi, Kamwenge, Kituza and Rukungiri had significantly younger coffee trees while Kawanda and Jinja trees were significantly older (Table 4.8). Tree girth as well was notably large in the Kawanda trees and smaller in trees from Kalangala, Kamwenge and Kituza. Tree production capacity was significantly lower at Entebbe botanical gardens and higher in the districts of Hoima, Kiboga, and Kyenjojo. Stem numbers were significantly few in Bushenyi, Kawanda, Kalangala, Kayunga, Kituza, whereas trees from Kibale district had numerous stems. Trees with significantly longer internodes were from Bundibudyo, Hoima, Jinja, Kawanda, Kabarole, Kiboga, Kibale, Kjenjojo, Kamuli, Kamwenge, Mukono and Masindi. Robusta trees with longer leaves were dominant in Entebbe, Iganga, Kawanda, Kalangala, Kituza, Mukono, Mbarara and Mayuge. Significantly broader leaf found in Kawanda, Kyenjojo, Kalangala and Mukono. Though not significant, fruit yields were low in all sites including research fields. Significantly reduced fruit yields were from Entebbe, Hoima, Jinja, Kawanda, Kabarole, Kiboga, Kibale, Kyenjojo, Kayunga, Kituza, Masaka, Mbarara and Rukungiri.

Table 4.8: ANOVA of phenotypic characters of 476 genotypes in 24 locations

ANOVA statistic summary							
Mxtr	**R^2**	**v.r**	**p v**	**location**	**m.c**	**s.e**	**p v**
Age	0.45	15.79	****	BS, JJ,	-15.36, 11.7	5.64, 5.71	****, ***
				KA, KW	36.2, -18.4	4.71, 5.26	**, ***
				KZ, RG	-23.8, -11.7	5.89, 5.71	****, **
Girth	0.27	7.24	****	JJ, KA	22, 39.6	9.67, 7.98	**, ****
				KL, KW	-19.0, 23.3	9.67, 8.90	**, **
				KZ	-22.9	9.97	**
PC	0.23	5.85	****	EB, HM	-1.52, 0.5	0.24, 1.9	****, ***
				KG, KJ	0.4, 0.52	0.2, 0.2	**, ***
Vigour	0.11	2.40	****	ns			
Stems	0.18	4.31	****	BS, KA	-3.28, -4.0	1.09, 0.91	***, ****
				KI, KL	-3.3, 2.5	1.06, 1.11	**, ***
				KY, KZ	-2.7, -4.1	1.1, 1.14	**, ****
IL	0.24	6.35	****	BU, HM	1.4, 1.56	0.35, 0.32	****, ***
				JJ, KA	1.21	0.34, 0.28	****, ****
				KB, KG	1.15, 0.8	0.34, 0.33	***, **
				KI, KJ	1.2, 0.72	0.33, 0.32	****, **
				KM, KW	1.0, 1.32	0.33, 0.32	**, ****
				MK, MS	1.45, 1.0	0.36, 0.38	****, **
LL	0.20	4.95	****	BG, EB	1.90, 1.99	0.78, 0.90	**, **
				JJ, KA	1.53, 2.45	0.77, 0.63	***, ***
				KL, KZ	2.16, 2.53	0.77, 0.79	**, ***
				MK, MR	1.74, -1.51	0.80, 0.76	**, **
				MY	1.81	0.77	**
LW	0.10	2.15	***	KA, KJ	0.75, 0.75	0.31, 0.35	**, **
				KL, MK	1.15, 1.35	0.38, 0.39	****, ***
Fruits	0.08	1.79	**	EB, HM	-3.8, -3.1	1.78, 1.38	**, **
				JJ, KA	-3.0, -2.7	1.50, 1.24	**, **
				KB, KG	-3.9, -3.3	1.50, 1.45	**, **
				KI, KJ	-4.9, -3.2	1.45, 1.42	***, ***
				KY, KZ	-3.5, -6.5	1.46, 1.55	**, ****
				MA, MR	-3.6, -4.0	1.50, 1.48	**, ***
				RG	-4.3	1.50	***
F Stat; F23, 452							

, *, **** significant at 0.05, 0.008, 0.0004 levels of probability

Key to Table 4.8: Mxtr=morphology character; R^2=coefficient of multiple determination; v.r=variance ratio; p.v=probability value; m.c=model coefficient; s.e=standard error; ns=not significant; PC=tree production capacity; IL=internode length; LL=leaf length. Refer to Table 4.1 for details of location code.

Multivariate analysis of morphological characters using the PCA is shown in Figure 4.6. Factor one of morphological parameter PCA represented 23.86% variance while factor two had a variance of 21.99%. The genotypes did not cluster into distinct diversity groups. The total morphological variance of 45.85% in the PCA was dominated by genotypes from Kamwenge and Kyenjojo (Western highlands region). Except for stems, all the other morphological characters were distributed on the positive side of the PC2 axis as shown in Figure 4.6. Productive, vigorous trees with many fruits were grouped together. Trees with long internodes, long and broad leaves clustered on their own. Old trees with large girth were together. The left hand negative section below PC2 comprised young trees with small leaves and short internodes as shown in Figure 4.6.

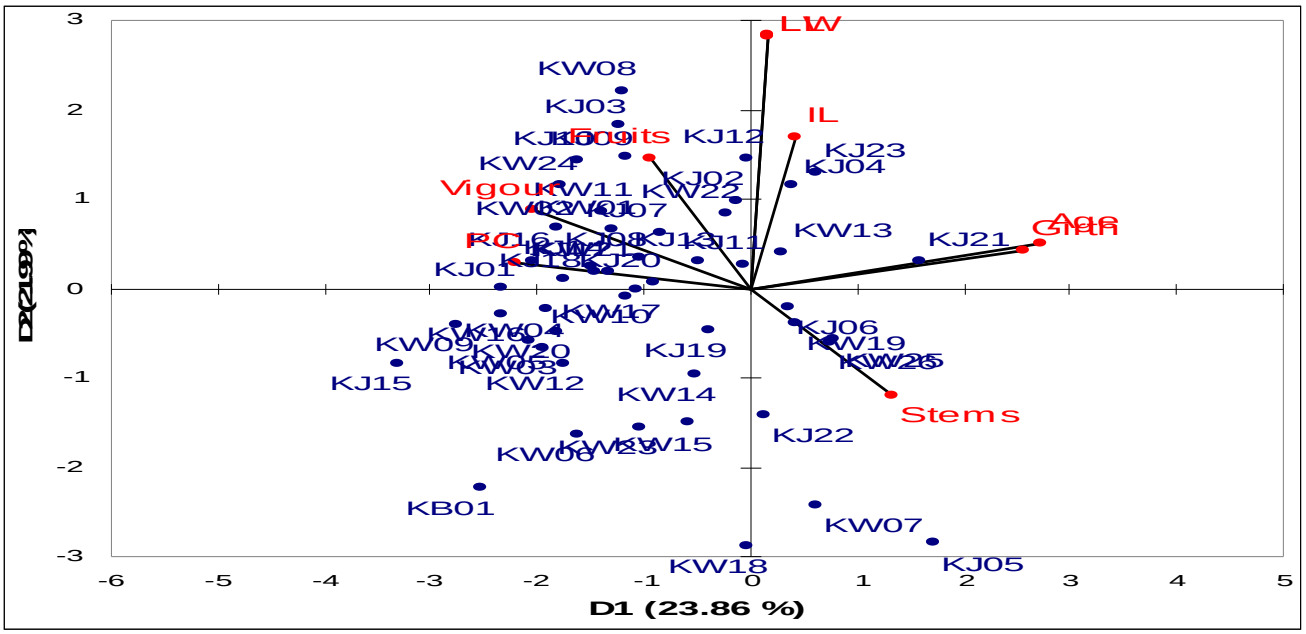


Figure 4.6: Principal component analysis of phenotypic parameters of 476 genotypes

97.42% morphology parameter variance was scored under factor 1 whereas factor 1 had only 2.58% (Figure 4.7). Group 2 was positioned between class 1 and 3. Group 1 comprised a majority of young, productive and vigorous trees with few stems. Class 2 had also some young vigorous and productive trees with long internodes, long and broad leaves with many fruits and stems. Group 3

was dominantly old trees. There was an apparent relationship between altitude with production capacity, vigour and number of fruits. The farthest groups were 1 and 3 and the nearest were 1 and 2.

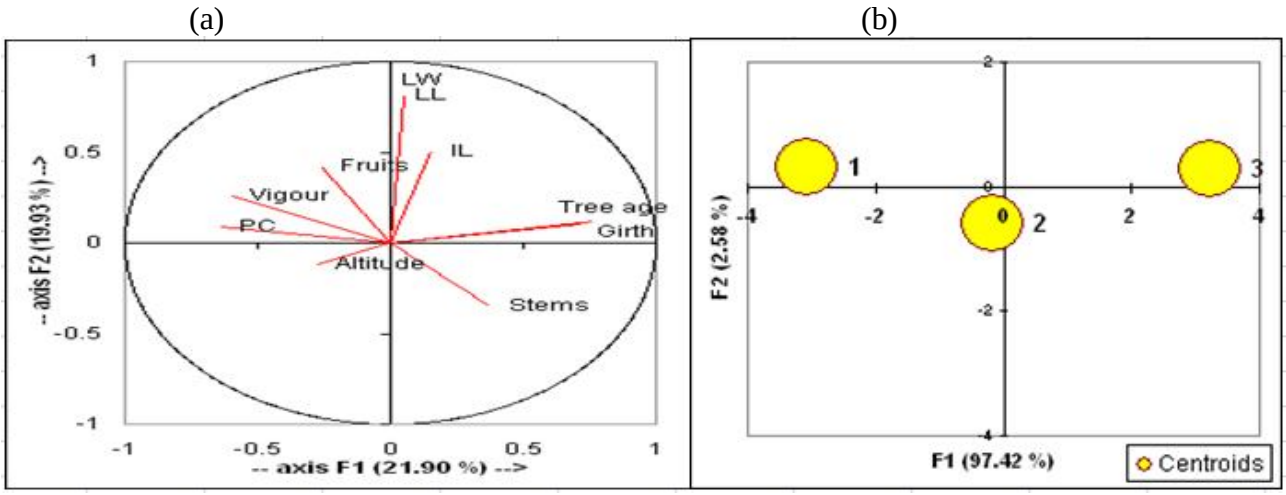


Figure 4.7: Relationships among a) morphological parameters with altitude b) morphological attribute groups

PC=production capacity (scale 1-5); IL=inter node length (cm); LL=leaf length (cm) LW=leaf width (cm); F=principal component

Correct placement of the different coffee types in right groups ranged from 6.82% (commercial) to 62.50% ("nganda") with an average of 49.79% (Table 4.9). Of the 27 trees initially rated as commercial types, only 3 were retained while 27 were regarded as "erecta", another 11 as "nganda" and 3 as hybrids. Retention of genotypes from multivariate morphological groups ranged from 91.77 to 97.77% with an average of 95.29% (Table 4.9).

Table 4.9: Estimate of 476 genotype group placement based on coffee type and morphological parameters using a confusion matrix

Coffee types							Multivariate morphology attribute groups					
From\ to	com	UE	UH	UN	total	% correct	from\ to	1	2	3	Total	% correct
com	3	27	3	11	44	6.82	1	219	5	0	224	97.77
UE	1	89	2	100	192	46.35	2	10	156	4	170	91.77
UH	0	6	5	5	16	31.25	3	0	3	79	82	96.34
UN	0	84	0	140	224	62.50	Total	229	164	83	476	95.29
Total	4	206	10	256	476	49.79						

Note: Rows = Given groups; Columns = Predicted groups

Key to Table 4.9: com=commercial UE="erecta"; UH=hybrids; UN="nganda"

The highest variance in group formation was from tree girth and age while the lowest was from tree vigour and production capacity (Table 4.10, first and second column). The commercial varieties had the most fruits, longest internode length and the highest production capacity at an average age of 12 years. "Erecta" types had the broadest leaves. Hybrids as were of average age 12 years coupled with the longest leaves, most vigorous trees and few fruits, stems, short and narrow leaves. "Nganda" types were the oldest with many stems, shorter leaves, low tree productivity and vigour.

In Table 4.10, group 1 had young trees (average 15 years) with few stems and high vigour and productivity. Values for fruits, leaf and internode length as well as leaf width were moderate under group 1. Group 2 consisted of moderate tree age (average 45 years) with fewer fruits, shorter leaf and internode length and leaf width. Stems were excessively many among group 2 genotypes. Group 3 was dominated by older trees of mean age 66 that had many fruits, long internodes, long and broad leaves. Tree vigour among old trees was low and the number of stems and production capacity was moderate.

Table 4.10: Pooled Robusta coffee morphological character contribution and mean group estimates derived from Bray-Curtis distance measure using 476 phenotypes

Traits	Cumulative % variance contribution		Mean group abundance						
			Coffee types				Multivariate groups		
	Coffee types	Multivariate groups	Com	UE	UH	UN	1	2	3
Girth	45.41	48.75	46.5	69.6	47	74.3	43.6	75	126
Age	78.93	83.64	11.7	36.9	12	37.4	15.2	43.5	65.5
Fruits	85.92	89.02	20.9	20.6	16.5	19.9	20.2	20	20.7
Stems	90.74	93	4.09	5.33	3.25	6.63	4.73	6.94	6.11
LL	94.59	95.9	19.2	18.3	19.9	17.9	18.4	17.7	19
IL	96.43	97.27	6.39	6.2	5.09	5.98	6.06	6.01	6.27
LW	98.19	98.62	7.38	7.47	6.96	7.36	7.39	7.34	7.5
PC	99.15	99.35	3.73	3.3	3.56	3.26	3.46	3.21	3.23
Vigour	100	100	3.55	3.22	3.56	3.18	3.32	3.18	3.16

Key to Table 4.10: UE="erecta"; UH=hybrids; UN="nganda"; PC=production capacity (scale 1-5); IL=inter node length (cm); LL=leaf length (cm) LW=leaf width (cm); F=principal component

One way analysis of similarity (ANOSIM) for Robusta coffee types and multivariate phenotypic character diversity groups revealed a mean rank within variance (5.5E04; 2.982E04), a mean rank between variance (5.8E04; 7.271E04) and R^2 of (0.04; 0.76) respectively (Figure 4.8). As in Bray-Curtis distance pair wise comparisons, the probability that the coffee types and multivariate groups were not the same was highly significant {P (same): 0.0001}. The median, inter quartile range and variance for "erecta" and "nganda" types were as high as that for between groups Figure 4.8a. The hybrids had the lowest variance and most stable with the lowest median. One way ANOSIM showed the mean, variance and range between groups was much higher than that observed within any of the phenotypic character diversity groups (Figure 4.8b). Group 1 had the highest mean, variance and inter quartile range while group 3 had the lowest records.

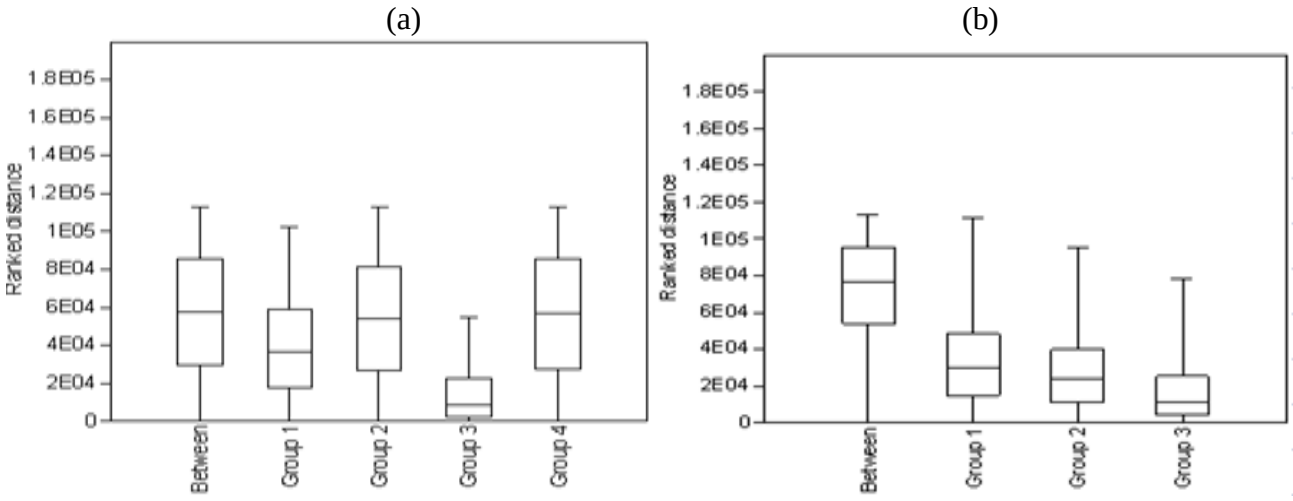


Figure 4.8: Description of (a) coffee types and (b) multivariate phenotypic diversity groups using ANOSIM box plots

Key to Figure 4.8a: 1=commercial; 2="erecta"; 3=hybrids; 4="nganda"

Robusta ripe fruit and green bean physical characters are shown in Figure 4.9. The ripe coffee colours were red, black orange, brown, purple and green. Common green bean colours were green, greenish, pale green, brown, brown grey and pale brown. Ripe cherry varied from very small to large whereas beans larger than screen size 20 (8 mm) and smaller than screen size 8 (3 mm) in diameter were found. Ripe and green bean shape variations included oblong, roundish and pointed. Ripe coffee fruit pulp ranged from sweet to unpalatable.

Figure 4.9: Diversity of Ugandan cultivated Robusta coffee ripe cherry and green bean shades

The least time used to roast a gram of green beans was 0.05 seconds while the longest was 0.38 with a mean of 0.11 (Table 4.11). Roasted green beans weight loss ranged from 4.30%-51.59% with an average of 15.29%. Percentage increase of roasted beans was 11.80-180.0% with an average of 70.98%. Green bean 100 seed weight ranged between 5.10-28.80 grams with an average of 14.55 grams. Some samples lacked green beans of screen 18 and 12. Some samples had 91.90% screen size 18 green beans with an average 19.03%. Average screen size 15 had a minimum value of 7.1% with a maximum of 94.10% and a mean of 61.27%. However, screen size 15 and above had a minimum value of 7.1% and a mean of 80.30%, with some accessions having all large bean size. The highest record for screen size 12 was 92.90% with a mean value of 19.87%.

Table 4.11: Variability of green bean physical characters for 204 Ugandan farm Robusta coffee genotypes

	RTPG	GB%WTdec	RB%VLinc	SDD	scr18	scr15	≥scr15	≤scr12
Minimum	0.05	4.3	11.8	5.1	0	7.1	7.1	0
Maximum	0.38	51.59	180	28.8	91.9	94.1	100	92.9
Mean	0.11	15.22	70.98	14.55	19.03	61.27	80.3	19.87
s.e	0	0.4	1.33	0.22	1.62	1.57	1.63	1.64

Key to Table 4.11: Altitude; age=tree age; RTPG=roast time per gram (seconds); GB=roasted green bean percentage weight decrease; RB=roasted green bean percentage weight increase; SSD=seed density; S18=screen size 18; S15=screen size 15; S≥15=screen size 15 and above; S≤12=screen size 12 and below; s.e=standard error.

Optimum height required to attain best bean physical characters was 1190 m above sea level at the age of 31 years (Table 5.12). Suitable altitude for dense beans was 1044 to 1334 m above sea level with tree age range 9-53 years. Suitable altitude range for screen size 15 and above beans was 1030-1336 m above sea level at age range 7-53 years. Altitude between 1041-1345 m above sea level had more screen size 12 beans produced by trees of 12-56 years.

Table 4.12: Gaussian gradient and tree age for cultivated Robusta coffee green bean physical characters

	RTPG	GB%WT decrease	RB%VL increase	SDD	scr18	scr15	scr≥15	scr≤12
Optmum alt	1183.9 ±146.0	1182.92 ±154.8	1188.54 ±151.07	1189.2 ±145.0	1198.1 ±125.1	1183.3 ±153.1	1186.8 ±147.1	1193.4 ±152.4
Maxm alt	0.38	51.59	180	28.8	91.9	94.1	100	92.9
Optimum age	32.9 ±22.3	30.5 ±21.5	31.7 ±21.7	31.3 ±21.9	30.3 ±23.1	31.3 ±20.9	31.1 ±21.5	34.1 ±21.9
Maxm age	0.38	51.6	180	28.8	91.9	94.1	100	92.9

Key to Table 4.12: alt= altitude; age=tree age; RTPG=roast time per gram (seconds); GB=roasted green bean percentage weight decrease; RB=roasted green bean percentage weight increase; SSD=seed density; S18=screen size 18; S15=screen size 15; S≥15=screen size 15 and above; S≤12=screen size 12 and below.

Clay loam soil texture produced Robusta coffee green beans that were significantly less dense with increased volume when roasted (Table 4.13). The "erecta" coffee types roasted longer. The commercial and hybrid types had high seed density and more beans of screen size 18 (Table 4.13). In addition to being dense, hybrid types had significantly fewer screen size 15 beans whereas the commercial types had more of screen size ≥15 and above (Table 4.13).

Table 4.13: Analysis of variance (ANOVA) of farm Robusta coffee green bean physical characters with soil texture and coffee types

ANOVA statistic summary								
Bxtr	**R^2**	**d.f**	**v.r**	**p v**	**Sdf**	**m.c**	**s.e**	**p v**
RB	0.05	2,201	5.05	***	clay loam	26.34	8.42	***
SDD	0.05	2,201	5.69	***	clay loam	-4.35	1.40	***
RTPG	0.04	3,200	2.49	*	erecta	0.01	0.006	*
SDD	0.23	3,200	19.88	****	com, hybrids	2.89, 7.12	0.70, 1.03	****, ****
S18	0.22	3,200	18.43	****	com, hybrids	22.9, 46.8	5.19, 7.60	****, ****
S15	0.08	3,200	5.83	**	hybrids	-32.24	8.0	****
S≥15	0.06	3,200	3.86	**	com	14.37	5.74	**

*, **, ***, **** significant at 06, 0.05, 0.007, 0.0001 levels of probability

Key to Table 4.13: Bxtr=bean physical character; R^2=coefficient of determination; d.f= degrees of freedom; v.r=variance ratio; p v=probability value; sdf=significantly different factor; m.c=model coefficient; com=commercial types.

The hybrids had denser and more screen size 18 beans than "nganda", "erecta" and commercial types (Figure 4.10a). "Nganda" types had the least dense beans with a few screen size 18 and more screen size 15. Clay loam soil had the highest roast bean volume increase with the least dense beans (Figure 4.10b). Beans from sandy loam soil were most dense. Loam soil had least bean weight and beans with the lowest roast bean volume increase

(a) (b) (c)

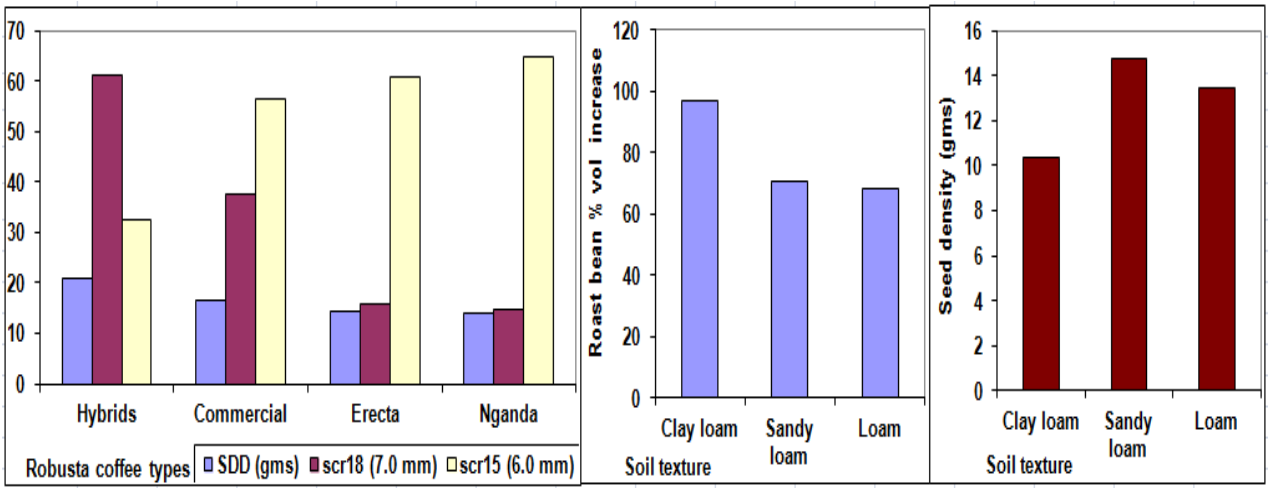


Figure 4.10 (a, b): Comparison of mean green bean physical characters in different coffee types and soils

Key to Figure 4.10: SDD=seed density; scr18=screen size 18; scr15=screen size 15

Robusta coffee trees of 4-40 years produced beans that significantly roasted for shorter time (Table 4.14). The highest amount of average screen size 15 beans was produced by trees of 4-10 and 21-50 age range. A significant reduction in roasted green bean weight was found in elevation 1101-1200 m above sea level. Altitude had a significant effect on seed density. Beans of 1401-1500 m above sea level had few screen size ≥15 with more screen size ≤12.

Table 4.14: ANOVA of farm Robusta coffee green bean physical characters with tree age and altitude

ANOVA statistic summary								
Bxtr	R2	d.f	v.r	p v	years	m.c	s.e	p v
RTPG	0.07	7,196	1.96	*	4-10, 11-20	-0.02, -02	0.01, 0.01	**,**
					21-30, 31-40	-0.03,-0.03	0.01, 0.01	***, **
S15	0.07	7,196	1.97	*	4-10, 21-30	14.78, 19.70	6.50, 7.04	**, ***
					31-40, 41-50	20.38, 15.48	7.84, 7.12	**, **
GB	0.06	6,197	2.07	*	1101-1200	-4.65	2.35	**
SDD	0.07	6,197	2.44	**				
S≥15	0.04	6,197	1.41	ns	1401-1500	-28.62	11.75	**
S≤12	0.03	6,197	1.03	ns	1401-1500	24.12	11.87	**

*, **, *** significantly different at 09, 0.05, 0.008 levels of probability.

Key to Table 4.14: Bxtr=bean physical character; R^2=coefficient of determination; d.f= degrees of freedom; v.r=variance ratio; p v=probability value; sdf=significantly different factor; m.c=model coefficient; com=commercial types; RTPG=roast time per gram (seconds); S15=screen size 15; GB=roasted green bean percentage weight decrease; RB=roasted green bean percentage weight increase; SDD=seed density; S≥15=screen size 15 and above; S≤12=screen size 12 and below.

Beans from Bushenyi significantly reduced in weight while those from Masaka had significantly reduced volume upon roasting (Table 4.15). Significantly dense and large beans of screen size 18 were collected from Kituza and Mukono. Mayuge also had significantly large beans of screen size 18. Coffee beans that were obtained from Kawanda and Kituza had significant low amounts of average screen size 15 beans. Green beans of above screen size 15 were significantly more in Kituza, Mukono and Rukungiri. Screen size 12 and below beans were significantly few from Jinja, Kituza, Mukono and Rukungiri.

Table 4.15: ANOVA of farm Robusta coffee green bean physical characters with locations

ANOVA statistic summary							
Bxtr	R2	v.r	p v	altitude	model coefficient	s.e	p v
GB	0.20	2.01	***	BS	11.69	2.42	****
RB	0.10	0.92	ns	MA	-19.13	7.62	**
SDD	0.26	2.94	****	KZ	7.68	1.29	****
				MK	2.97	1.20	**
Scr18	0.27	3.11	****	KZ	52.4	9.4	****
				MK, MY	23.85, 47.4	8.78, 13.36	***, ****
S15	0.22	2.27	***	KA, KZ	-17.3, -31.98	7.87, 9.47	**, ***
S≥15	0.15	1.49	*	KZ, MK	20.4, 19.5	10.2, 9.6	**, **
				RG	22.33	9.9	**
S≤12	0.18	1.75	**	JJ, KZ	-19.2, -21.8	-26, -22	**, **
				MK, RG	21.5, -21.9	9.5, 9.8	**, **
Fstat; F22, 181							

, *, **** significant at 0.05, 0.007, 0.0005 levels of probability

Key to Table 4.15: Bxtr=bean physical character; R^2=coefficient of multiple determination; v.r=variance ratio; p.v=probability value; m.c=model coefficient; s.e=standard error; ns=not significant; age=tree age; RTPG=roast time per gram (seconds); GB=roasted green bean percentage weight decrease; RB=roasted green bean percentage weight increase; SDD=seed density; S18=screen size 18; S≥15=screen size 15 and above; S15=screen size 15; S≤12=screen size 12 and below; Refer to Table 4.1 for details of location.

Four physical green bean character groups were formed (Figure 4.11). Beans of screen size 12 and below had more roast time with a high roast bean volume increase. Beans mainly from Hoima and others from Bushenyi, Iganga and controls (CN01 and CN02) had more roast time with a high roast bean volume increase. Dense beans were associated with bean size greater than screen size 15 and were mainly from mainly from Bugiri, Jinja, Bundibudyo, Hoima, Bushenyi and control CN03 (Figure 4.11). All samples underwent a reduction in green bean weight upon roasting.

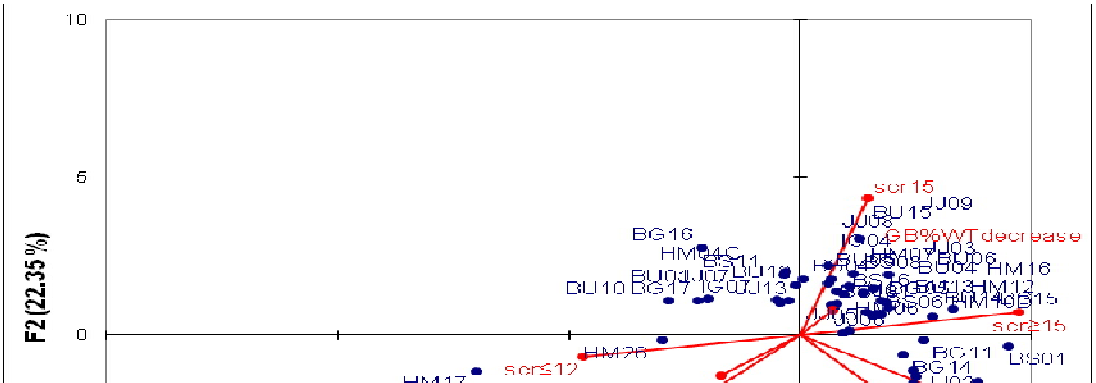
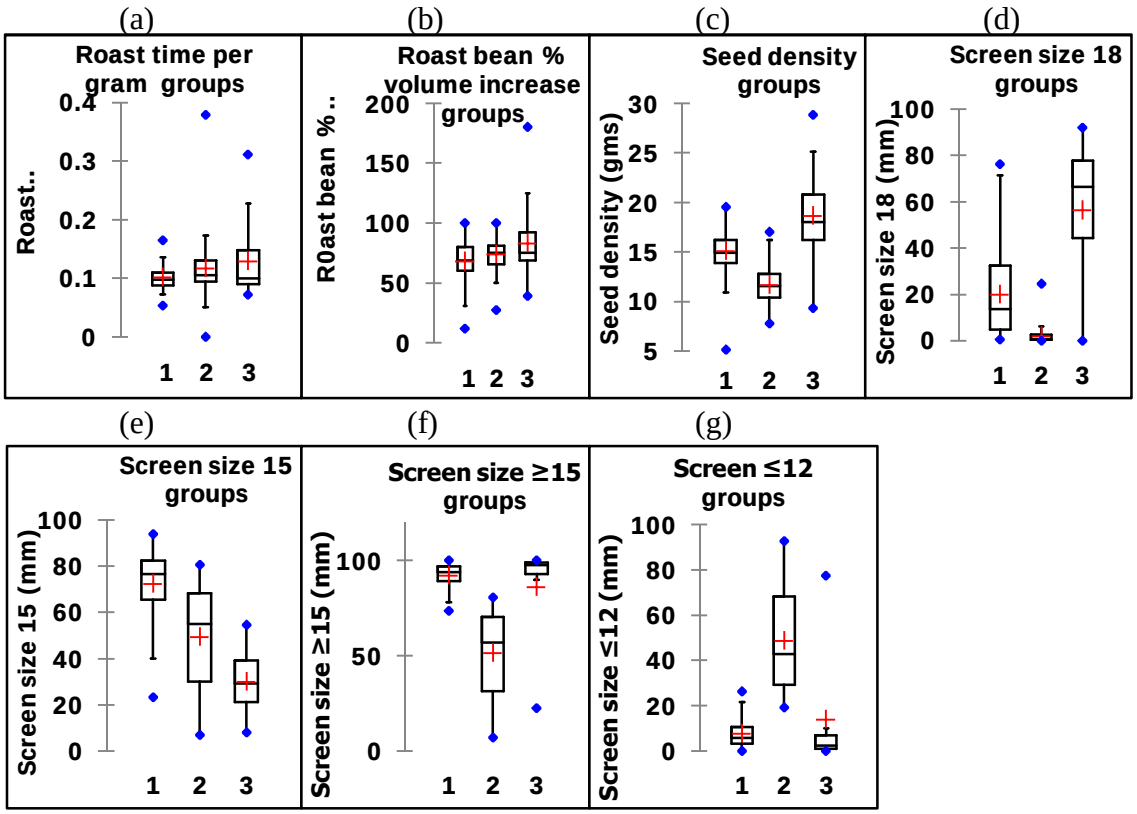


Figure 4.11: Spatial presentation of farm Robusta coffee physical green bean character diversity using a principal component analysis

Key to Figure 4.11: RTPG=roast time per gram (seconds); GB%WT decrease=roasted green bean percentage weight decrease; RB%VLincrease=roasted green bean percentage weight increase; SDD=seed density; S18=screen size 18; S≥15=screen size 15 and above; S15=screen size 15; S≤12=screen size 12 and below.

Box plot descriptions of bean physical characters are shown in Figures 4.12 (a-g). Descriptions Group 3 had consistently high values for mean, variance and inter-quartile range in roast time, roast bean volume increase, seed density and screen 18 attributes (Figure 4.12a-d). Group 2 variance and inter-quartile range was high for screen size 15, screen size 15 and above and screen size 12 and below (Figure 4.12e-g). Attribute group means, median, variance, inter-quartile range were variable. Group mean values were either greater, lower or equivalent to the median.



Figures 4.12 (a-g): Descriptions of green bean physical character diversity groups using box plots

In both Mahalanobis and Fisher distances, the largest distance was between group 1 and 3 while the shortest was from 1 to 2 (Table 4.16). Mahalanobis distances of greater than 3 indicated that the three groups were different. All Fisher probability values were highly significant.

Table 4.16: Distances between green bean physical character diversity groups

Group	Mahalanobis distances			Fisher distances			p-values for Fisher distances		
	1	2	3	1	2	3	1	2	3
1	0	10.01	5.49	0	55.29	30.32	1	<0.0001	<0.0001
2	10.01	0	13.26	55.29	0	73.25	<0.0001	1	<0.0001
3	5.49	13.26	0	30.32	73.25	0	<0.0001	<0.0001	1

Association between bean physical characters, altitude and multivariate bean physical attribute groups is illustrated in Figure 4.13 a, b. Group 1 that had a positive association with younger trees had a negative correlation with roast time, elevation but positively with roasted green bean weight loss. Aged trees were correlated with group 2 that related to dominantly small beans of screen size 12 with a high roast time, volume and weight loss. Group 3 beans comprised dense and larger beans of screen size 15 and above (Figure 4.13a). Screen size 15 and above had a few screen size 12 and below beans. Screen size 18 beans had a positive correlation with dense beans. High altitude was correlated to increased bean roast time, seed density and screen size 15 to 18. The furthest distance was between group 2 and 3 while the shortest was between 1 and 3.

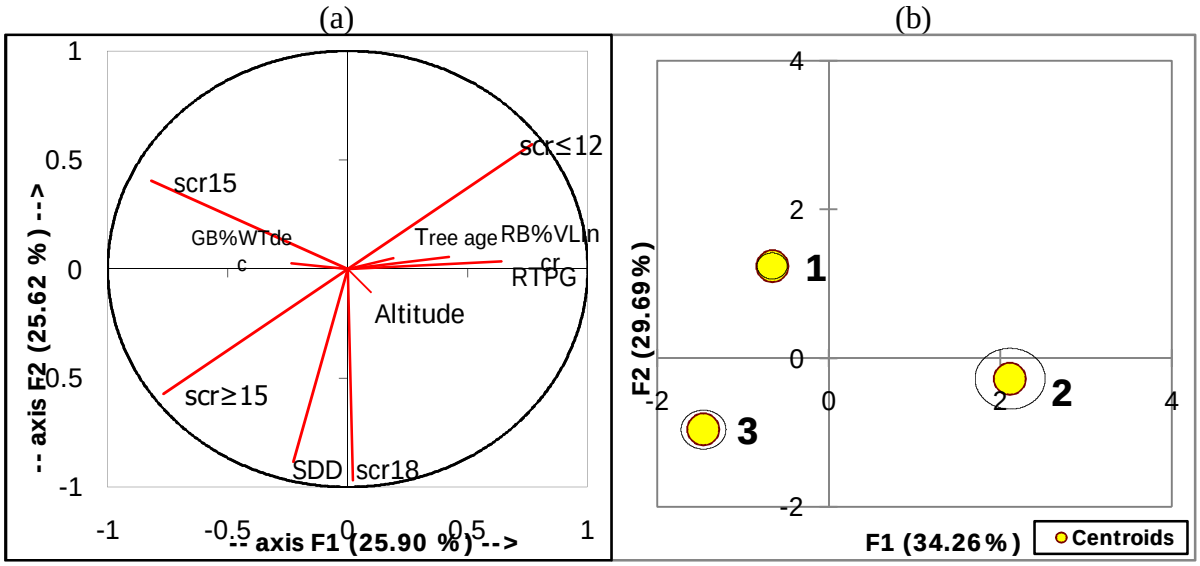


Figure 4.13 a, b: Green bean physical character (a) relationships from the principle component analysis (b) diversity groups from factorial step discriminant analysis.

Key to Figure 4.13: RTPG=roast time per gram (seconds); GB%WT decrease=roasted green bean percentage weight decrease; RB%VLincrease=roasted green bean percentage weight increase; SDD=seed density; S18=screen size 18; S≥15=screen size 15 and above; S15=screen size 15; S≤12=screen size 12 and below.

Correct genotype placement ranged between 58.62-92.45% with a mean of 82.44% (Table 4.17). The farthest groups were 2 and 3 while the shortest was from 1 to 2.

Table 4.17: Estimated genotype class placement and pair wise distance comparison for green bean physical characters using a confusion matrix.

Confusion matrix					
from \ to	1	2	3	Total	% correct
1	115	0	10	125	92.45%
2	10	45	1	56	77.14%
3	3	1	19	23	58.62%
Total	128	46	30	204	82.44%

Group 1 was characterized as having dense beans of screen size 15 and above with a high weight reduction upon roasting (Table 4.18). Screen size 12 and below grade beans that had a high volume increase when roasted were fewer in group 1. Group 2 had mainly screen size 12 with a few dense beans. Dense beans of screen size 18 whose volume increased during roasting were categorized under group 3, and had a few average screen size 15, minimal weight loss when roasted and highest roast time.

Table 4.18: Similarity percentage for pooled Robusta coffee green bean physical characteristics from Bray-Curtis distance measure

Pooled groups	Variance		Mean group abundance		
Taxon	Contribution	Cumulative %	1	2	3
Screen size ≥15	6.46	22.27	92.2	51.4	86
Screen size ≤12	6.46	44.54	7.8	48.6	14
Screen size 15	5.76	64.39	72.3	49.5	29.9
Screen size 18	5.18	82.26	19.9	1.88	56.1
RB%VLincrease	3.74	95.16	67.6	73.6	83
Seed density	0.80	97.91	15.1	11.6	18.6
GB%WTdecrease	0.60	99.98	16	14.4	13.2
RTPG	0.01	100	0.10	0.12	0.13

Key to Table 4.18: RB%VLincrease=roasted bean % weight increase; GB%WT decrease=roasted bean % weight decrease; RTPG=roast time per gram (seconds).

In Figure 4.14, One way multivariate analysis of similarity (ANOSIM) revealed a within population mean variability of 6729, between population variance (1.3E04), R (0.65) and probability value of (P = 0.0001) as was also found in pair wise comparisons using Bray-Curtis distances. The highest mean was between groups (Figure 4.14). Group 1 with dense beans of screen size 15 and above had the least mean and variance and hence was more stable. Category 3 that had dense beans whose volume increased a lot more when roasted was the most variable. Smaller beans of screen size 12 and below had the second highest mean and variance.

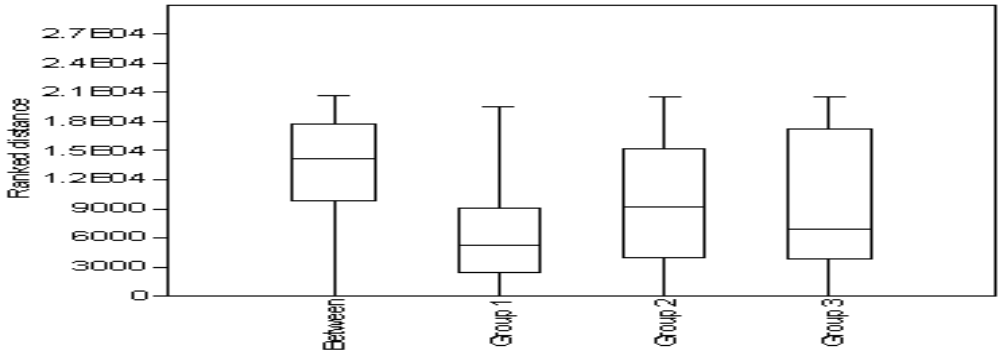


Figure 4.14: Analysis of similarity (ANOSIM) for green bean physical characters using box plots

4.4 Discussion

Tree morphological characters could not be grouped into distinct diversity groups using the principal component analysis as shown by Figure 4.6, but were categorized into groups using the multivariate analyses (Table 4.10; Figure 4.7). Morphological differences in evaluated genotypes might have been too small to be detected or masked by the interactive effects of genotype and environment that the PCA could not separate. Other researchers also reported that genetic diversity using phenotypic traits revealed low species organization with the PCA and intra-specific variations in vegetative traits especially leaf and fruit characters were not detected (Montagnon et al., 1996; Leroy et al., 1993). From the present morphological diversity results, farm management practices

seemed to have contributed greatly to coffee type and multivariate group formation (Table 4.10). For instance, one distinct group that had young trees of average 15 years had few stems, high vigour and productivity with moderate fruits, leaf and inter node length as well as leaf width (Table 4.6).

Effect of tree age coupled with poor canopy management was apparent in group 2 that comprised 45 old year trees that had many stems resulting to fewer fruits. Genotype and environment interaction could have induced shorter internode, short and narrow leaves realized in group 2. In the third group it became apparent that the dominantly old trees of average age 66 had reduced tree vigour although with many fruits, long internodes, long and broad leaves with moderate stems and production capacity. Old trees have a characteristically large open canopy that exposes many nodes to sunlight inducing much flowering and bearing of many fruits. Excess shade induces etiolated plants with long internodes and big leaves to increase chance of trapping sunlight.

It is also likely that extractable information from other morphological characters was minimized by the high variance that physiological tree age range contributed to group formation (Table 4.10). In addition environment factors such as altitude (Figure 4.8) and location (Table 4.8) influenced morphological trait expression. The fact that genotypes with high phenotypic attribute values originated from different locations in varying numbers as shown in Figure 4.6, points out a possibility of individual genotypes contributing to this variability, similarly like the genetic diversity (Table 3.10).

PCA analysis also revealed that most phenotypic diversity was from the Western highlands of Kamwenge, Kyenjojo, Bundibudyo, Kabarole and Hoima in Lake Albert Crescent region (Figure 4.6). Farmer cultural norm of collecting volunteer planting materials from neighbouring forests such as Kibale, Itwara, Bugoma, Bwemoa and Budongo (Figure 3.1) could have contributed to the

high phenotypic variability in these regions since they are near the natural forests. In some cases, land races would be distributed and planted as pre-selections of farmer choice. Neighboring farmers tend to share planting material or source from a common locality more than with distant areas. Farmers also narrated that they inherited some Robusta coffee plots from previous generations that thinned out forest tree species leaving Robusta coffee trees in their natural setting. Phenotypic divergences among and within land race populations could also be related to geographical origin or isolation.

The results here further verified that large leaves were correlated with the erect habit while narrow shorter leaves associated with the spreading "nganda" (Table 4.3). Selection of highly segregating progenies could have contributed to the characteristic long and narrow leaves with the shortest internodes and least fruits observed from the hybrids. The commercial types that were closely related to erect types had the longest internodes, highest production capacity, most fruits, longest and broadest leaves (Table 4.3, Figure 4.6; Figure 4.6a,b) an indication that conscious phenotypic selection for these traits might have been practiced. The big leaves provided ample photosynthetic area to nourish tree vegetative parts and fruits. The long internodes offered adequate space for fruit nodes. Free formation of secondary branches on primaries stems, especially on erect trees with big leaves gives greater leaf area and more wood to bear flowers.

According to Wrigley (1988), shoot elongation in coffee was influenced by small temperature changes while soil moisture affected internode elongation. Furthermore, node production was assumed favorable during hot sunny conditions. In Figure 4.2b, the young (over 65%) and mature leaves (80%) green leaves were photosynthetic whereas the 35% bronze leaves may have been for tolerance to drying winds (Maitland, 1926; Thomas, 1935). The "nganda" had the most bronze leaf flush colour followed by "erecta" whereas light bronze leaf flush and green leaf colour was

common to all coffee types (Figure 4.2). The 12% light green leaves might have had less chlorophyll distribution and photosynthesis than the 8% dark green leaves. The 25% leaves with flat surface may experience more transpiration than those with 70% intermediate undulations (Figure 4.2).

Numerous stems were more in the "nganda" (Figure 4.7 a, b) and among trees of 11-70 years (Table 4.6). Many stems affect the distribution and utilization of light. Most plant nutrients also get diverted to sustain the many stems and branches consequently lowering tree productivity, vigour, yield, fruit size and density. The management of stems is exemplified by the fact genotypes with many stems had significantly short and narrow leaves and few fruits while trees with long internodes had long and broad leaves with many fruits (Figure 4.7a, b). Selective pruning of primaries regulates the leaf/fruit ratio and with adequate application of manure reduces die back and defoliation. Studies by Tiago et al., 2012 indicated that variation on yield was much correlated with variation of vegetation indices of the previous year. Infection by diseases or pests reduces tree productivity and yield.

With the "nganda" (48%) and "erecta" (40) landraces being the dominantly cultivated coffee types in Uganda as shown in Figure 4.2a, the phenotypic characteristics of cultivated Robusta coffee would be dominantly of the two coffee types. Earlier reports also indicated the spreading "nganda" and the upright "erecta" land races as the two distinctive Robusta coffee varieties grown in Uganda (Maitland, 1926; Thomas, 1935). Over 75% of farm trees had cylindrical shape (Figure 4.2a), usually attained over years when heavy tree yield pulls down and spread the primary and secondary branches. The spreading nature and open tree growth of "nganda" types is ideal for small scale farmers because they require minimal pruning after initial training, little weeding, the large canopy keeps the soil cool and encourages extensive root development (Thomas, 1947).

"Nganda" types were linked to vigour, and tolerance to leaf rust and production of secondary branches with large flower bracts (Wrigley, 1988). With good crop husbandry and consideration of primary length, the 1% dwarf trees (Figure 4.2a) and hybrids that were noted to be shorter than other coffee types can be planted at a higher population density to increase yield. The 47% estimated trees with thick and strong stems were likely to be the "erecta" types, characteristically known for strong stems, pale large leaves and bigger berries (Thomas, 1940; Thomas, 1944; Thomas, 1947). Strong stems ably carry fruit loads, although they tend to break with excessive yield. The 3% conical shaped trees (Figure 4.2) would be desirable for areas prone to snow or hail storms as the tree shape minimizes frozen water from settling on tree canopy.

While "nganda" and "erecta" types were the most variable coffee types because they had the largest mean value ranges (Table 4.3) and variance (Figure 4.11a), the difference between the two types was small when clustered with the PCA (Table 4.9; Figure 4.4) making their identification in the field difficult. Accurate placement of "erecta" and "nganda" types was only realized at 46.35% and 62.50% respectively as given in Table 4.9. It is likely that some genotypes re-grouped in either "nganda" or "erecta" as shown in Table 4.9 were their progenies. The cultivated "nganda" and "erecta" may then be assumed as hybrids of wild type "nganda" and "erecta". The high variability and close relatedness of "nganda" and "erecta" would result from self in-compatibility in cultivated mixtures of the landraces (Maitland, 1926; Leakey, 1970) that generate variability through hybridizing genetic units producing progeny with traits of either parent. Separating the "nganda" and "erecta" types using traits such as stem strength, canopy shape, leaf size and colour is difficult as the traits get moderated by crop husbandry and environment. Poorly managed trees are either weakly looking or overgrown to have thick stems and cylindrical canopies that are difficult to differentiate (Wrigley, 1988). Most old trees were found at 1101-1200 m above sea level (Figure

4.7a), a height of rapid tree growth characterized by high temperatures and possibly reduced population pressure on Robusta coffee genetic resources as compared to areas at higher elevation.

The low 6.82% correct identification of commercial types at farmer fields (Table 4.9), instead of 100% (since salient characteristics of each variety should have been evident before release) could have resulted from the fact that some elite materials were distributed to farmers as seedlings (Kibirige-Sebunya et al., 1993; Kibirige-Sebunya et al., 1996). Segregating seedling planting material could deter identification of specifically known cultivar trait as observed by more than 50% re-grouping of the recorded commercial varieties as "erecta", a close relation as given in Table 4.9; Figure 4.6b, while another third was relocated to "nganda". In the case of the hybrids, 31.25% were placed correctly while similar percentages were relocated to "erecta" and "nganda" types. These two scenarios of results therefore suggest that the commercial and hybrid types were possibly selections from "erecta" and "nganda" progeny. On average, correct placement of genotypes in the right categories was estimated at 49.79% (Table 4.9), implying there was much more phenotypic variability in farm *C. canephora* than recorded and that identifying different coffee types using morphological traits was not straight forward. Genotype responses to different environments were likely to influence tree morphology.

The most unrelated morphological coffee types were the hybrids and landraces, particularly with "nganda" types (Figure 4.7b). The heterogeneous nature of landraces generates highly segregating progenies that provide vigorous hybrids as reported by Bertrand et al., 2005a. The commercial varieties were closer to the "erecta" types than "nganda" types (Figure 4.7b) possibly because were developed using a notable line No.9 collection from Toro that was believed to be "erecta" type (Wrigley, 1988; Kibirige-Sebunya et al., 1996). The commercial and hybrids were not very close

which is in agreement with the genetic diversity results that indicated that the hybrids and commercial types belonged to different diversity groups of cultivated Robusta (Figure 3.6; Figure 3.7).

By disregarding coffee type identities, group retention using three phenotypic diversity groups increased from 91.77 to 97.77% with an average of 95.29%, reflecting higher chances of correct placement (Table 4.9). Meanwhile the 9% commercial variety occurrence in farmer fields (Figure 4.2), since 1970s (Kibirige-Sebunya et al., 1993) indicates the slow adoption of new Robusta coffee technologies. Farmers possibly prefer landraces because they vary in response to hardy abiotic and biotic stress increase chances of not losing the crop completely during unfavorable conditions. Shortage or inaccessibility of research improved variety planting material contributed to limited availability of elite materials in farmer fields. It is easier for farmers to access free plantlets from the wild or land races from fellow farmers.

As a guide to farmers on economics of tree productivity, findings indicated that the “nganda” and “erecta” types whose total field occurrence was 88%, age range of 4-80 years with an average of 37 years and, had the most stems with low tree vigour and production capacity (Figure 4.7). Trees of 4-30 age were significantly productive while trees of 4-20 years were significantly vigorous (Table 4.6). With over 75% of farm coffee trees having cylindrical shaped with many tall stems (Figure 4.2a) and occasional narrow leaves in 41-50 and 61-70 (Table 4.6), it was not alarming to find out that at varying measures, fruit yields were low in all study locations including research fields (Table 4.8).

Unlike Kawanda where old trees were maintained as genetic resources, Jinja farmers had significantly old trees that were may have been economically unproductive (Table 4.8). Unlike the present results that estimated 21.2% cultivated Robusta coffee trees were up to 10 years, UCTF,

2008; 2009 reported 40% of the trees were less than 10 years of age. According to Wrigley (1988), age modifies tree growth habit in that fruiting nodes on the tree increased with age while fruits per node decreased. For young trees, yield was associated with the number of nodes while the number of fruits per node reflected yield in old trees. Cambrony, 1992 reported that yield increased from three to eight years when it stabilized but decreased after 15 years. Insignificant differences due to tree old age have been associated with inefficient use of nutrients by small headed primaries (Onzima et al., 1996). Furthermore, old tree primaries tend to become etiolated with self-pruned lower primaries and short internodes that provide little space for fruiting, consequently low yields, regardless to inorganic fertilizer application (Naakubuza et al., 2005).

Other environmental factors like soil texture, tree spacing, shade and altitude could affect tree yield. About 90% of study farms comprised sandy loam soil texture. Preferably coffee grows in well aerated, deep, well drained, neutral acidity in nutrient rich volcanic soils, although in Uganda climate has more influence than soil because Robusta grows even on soils that are red sandy, clay or gravely loams (Wrigley, 1988). Unfortunately, farmers reported that they hardly mulch or apply fertilizer, herbicides or pesticides although Ngambeki et al., 1992 indicated that 20% of farmers applied fertilizer in coffee production. Estimated peasant average yields of 600 kg/ha was only 10-30% of what could be achieved potentially (UCTF, 2008: 2009). In this work, 80% of the farms were randomly spaced as also reported by Butt et al., 1970; Thomas, 1935; 1940). Under moderate management, an average yield of 2,500kg/ha is obtainable with a recommended spacing of 3 meters by 3 meters from research commercial varieties (Kibirige-sebunya et al., 1996). Because some plants were inherited when at random spacing or before 3 meters by 3 meter spacing recommendation, subsequent planting of shade trees and intercrops required ample space co exist with Robusta coffee trees.

The 90% shade trees were of diverse species that were either vegetable, fruit (wild berries inclusive), legume, cereal, root crop or medicinal purpose. Shade decreases tree productivity by about 20% and induce a cool micro climate that prolongs bean filling producing fully developed beans (Vaast et al., 2006). Reduced tree annual bearing enables coffee to yield each year and farmer to reap continuously. Shade minimizes nutritional deficiencies by adding plant debris. Avoiding excessive shade reduces parasite attacks. Profuse flowering that normally occurs after a dry spell of high temperatures and sunshine is moderated by shade producing fewer beans.

Tree production capacity and vigour increased with elevation as indicated in Figure 4.11 up to a height of 1301-1400 m above sea level (Figure 4.5b). High leaf to fruit ratio reported in higher elevation arises from a longer leaf life span (Vaast et al., 2006) and increased bean nutrient supply. Delayed berry flesh ripening in low temperatures experienced at higher elevations, allowed for longer and better bean filling as reported by Vaast et al., 2006. However, the results here showed that Robusta coffee trees at 1101-1200 m above sea level significantly reduced tree vigour (Table 4.7). At lower altitude characterized by high temperatures, fast Robusta coffee growth subsequently made trees become less vigorous and less productive. The slow growth rate at higher altitude would generate fewer stems, shorter internodes and leaves with fairly younger looking trees than their counterparts at lower elevation observed in the study (Table 4.7; Figure 4.7a). Shorter internodes could provide less fruiting space while short leaves minimized photosynthetic surface. Tree productivity and vigour at height of 1401-1500 m above sea level was significantly reduced due to possible “heat and cold effect” that is common in cooler areas and affects photosynthetic rate.

The high variability observed in cultivated Robusta coffee ripe and green bean shades, size, weight, shape, structure (Table 4.11; Figure 4.8) and smell, suggests that Uganda is within the centre of origin (Thomas, 1940; Berthaud, 1986; Leakey, 1970; Musoli et al., 2009). Ugandan Robusta coffee is

widely distributed with strong adaptation to rainfall and soil types (Maitland, 1926; Thomas, 1940; Leakey, 1970). While the Principle Component Analysis estimated four green bean physical character diversity groups (Figure 4.11), multivariate analyses attained three categories (4.18; Figure 4.12; Figure 4.13; 4.14). Beans from one group were of mainly screen size 15 and above, with the shortest roast time and high roast weight loss. The short roast time observed from large beans in group 1 indicated that the beans though large were possibly inadequately developed in nutrient deposits, consequently high roast bean weight loss. The lowest mean and variance values realized in group 1 reflected a high level of homogeneity among group genotypes and their overall inferiority as regards to bean physical attributes. Group 2 beans had more screen size 12, less dense with moderate roast time, originating mainly from old trees.

Characteristics of group 2 beans point out an important aspect of inadequate farm management such as maintaining old trees that generate light small beans that sell cheaply. Category 3 that had dense beans of mainly screen size 18 with a high roast time, reduced roast weight loss, high roast volume increase and most variable provided for potential profits from bean weight, volume and quality. Genotype and good tendering contribute to improved bean quality. The wide mean bean physical trait ranges (Table 4.11) and bean size variance that dominated multivariate group formation (Table 4.18), implied a wide variation of gradable bean sizes existed in farms which can be channeled to diverse market demands. The out crossing nature of the species, the dominantly cultivated and widely distributed wild and landraces possibly contributed to the high bean variability (Maitland, 1926; Thomas, 1935). Other processes like evolution, farmer complex selection pressures and highly variable biophysical adaptation systems could generate variability. It is also possible that physiological disorders associated with old trees may induce some phenotypic variations.

Uganda's cultivated Robusta coffee comprised 61% average screen size 15 (Table 4.11). It is likely that the "nganda" types that constituted 48% of cultivated Robusta coffee (Figure 4.2) and had mainly screen size 15 beans (Figure 4.10a) significantly contributed to overall screen size 15. The 19% of screen size 18 and 20% of small screen size 12 and below (Table 4.11) could have been contributed by all coffee types since estimated frequency of commercial and hybrid types was 12%. Other than bean size the "nganda" and "erecta" land races were generally less productive as compared to commercial and hybrid types (Table 4.3; Figure 4.4a, b), because farmer selection criteria on landraces possibly considered tree adaptation to adverse management practices and environment conditions than yield and other desirable traits. The overall Robusta coffee seed density of 14.55% (Table 4.11) was within the range reported by Clarke, 1985.

Even without grading and separating each sample according to bean size before roasting, the research was able to detect that sample roast time depended on seed density and screen size and tree age (4.12). Dry green bean physical characters such as uniformity, size, density, shape and colour determined how long and evenly the coffee roasted. Large and dense beans roasted a lot longer (Figure 4.13a). Denser beans require a considerably darker roast in order to achieve similar flavour development and roast colour after grinding than lighter beans (Clifford et al., 1985). Screen size 15 beans, generally produced by younger trees roasted for a shorter time with a high bean weight loss (Figure 4.13a) possibly because of inadequate bean filling caused by overproduction. A high bean weight loss could also arise from excessive water loss and burning off of bean tissue, particularly with prolonged roast time Clarke, 1985.

Uneven bean colours normally hint drying problems, whereas different shapes indicate a possible mixture of varieties (Leroy et al., 2006). Overall, mean green bean percentage decrease and increase upon roasting were 15.22% and 70.98% as shown in Table 4.11 that was comparable to

other studies that reported an average of 16% weight loss and 50-80% roast volume increase (Clarke et al., 1985). The prolonged roast time observed in "erecta" beans (Table 4.13b) could be attributed to over productive nature of the type producing bold beans (Thomas, 1940: Thomas, 1944; Thomas, 1947) that could result in heavy beans under favourable conditions or less dense beans when conditions are unsuitable. Protracted roast time of "erecta" type can be caused by either less dense (Table 5.2) or large dense beans (Figure 5.6).

Beans from high elevation roasted longer, denser, of mainly screen size 18 and some beans greater than screen size 15 (Table 4.18; Figure 4.13). Increased carbohydrate supply to berries and higher bean fat synthesis at high altitude could have arisen from a longer leaf life span that increases leaf to fruit ratio reported in higher elevation by Vaast et al., 2006. Consequently, the delayed berry flesh ripening at low temperatures encountered at higher elevations, might have allowed for longer and better bean filling. At height of 1401-1500 m above sea level, trees produced smaller sizes with few large beans of screen size 15 and above as shown by Table 4.14b. At high altitude coffee beans tend to be greenish in colour, small, denser and harder (Clifford et al., 1985; Clarke, 1985) and possibly that is why some screen size 12 beans roasted longer with a high volume increase (Figure 4.11).

Similarly coffee trees at elevation 1101-1200 had significantly reduced vigour (Table 4.11) but higher roast green bean weight loss (Table 4.14) as observed by Vaast et al., 2006. Shorter internodes and leaves encountered at elevation 1401-1500 m above sea level reduce tree productivity and vigour consequently producing more screen size 12 and few screen size 15 (Table 4.14). High temperatures at lower altitude increase tree growth rate, consequently reducing tree vigour. Meanwhile beans may poorly develop at high altitude as a result of cold and warm air

distorting and discoloring the leaves. Like in other crops, hot and humid conditions yield lower flavour and bean structure.

Beans from 4-40 year old trees with a short roast time (Table 4.14) were possibly large beans of average screen size 15 and above (Figure 4.10; Figure 4.13). The significantly reduced bean roast weight loss observed in collections from Bushenyi and Masaka (Table 4.16) could have resulted from average screen size 15 beans of reduced density collected from younger trees. The dominant production of screen size 12 beans with a high roast time and volume by old trees (Figure 4.11) might have resulted from stressful conditions associated with advanced tree age that induce the production of lignified substances that roast for a long time.

High roast bean weight loss in young trees as in Figure 4.13 possibly arose from inadequate bean filling during development, which reduced bean size and dry matter. The stressful condition associated with overproduction coupled with inadequate nutrient element availability might have induced the production of lignified substances that require prolonged roasting. Based on Scanning Electron Microscope (SEM), Wang 2012 indicated that formed and ruptured pores associated with increased roast bean volume were likely to be much more in poorly developed beans. Unroasted coffee beans had relatively compact morphology while the heated beans had thin polyhedral walls surrounding the pores whose shape was determined by carbon dioxide pore pressure that against each other rupturing some pore walls. Specialty markets prefer coffee green beans with same origin, homogeneous size, colour, shape, species clean green bean smell, with a maximum moisture content of 12.5% (Leroy et al., 2006).

Factors such as genotype, tree physiological age, picking period, fruits to leaf ratio strongly influence the physical and chemical content of green beans (Vaast et al., 2006). Bean size was one

of the selection criteria for the hybrids and commercial types that made their beans significantly larger than landraces (Table 4.11; Figure 4.10a, b). As noted, physiological tree age and altitude as seen in Table 4.14 and location shown in Table 4.15 influenced bean size. Bean sizes have also been associated with the extent of perisperm development, which is more pronounced in shade grown than sun grown coffee trees (De Castro et al., 2008). The high ratio of reducing sugars to sucrose experienced over a longer period in shade than in sun grown coffee encourage cell division and elongation in young perisperm and endosperm tissues resulting into larger beans (Muschler, 2001; De Castro et al., 2006; Vaast et al., 2006). The seed physiology and development seemed to vary based on the position of the fruits within plagiotropic branches and the light received (Vaast et al., 2006), reflecting environmental effects on different gene expression, and quality of fruits derived from the same plant.

Furthermore, yellow or green cherries picked at the end of the picking season develop better quality characteristics such as larger beans and deposit more biochemical compounds as a result of prolonged bean growth (Muschler, 2001; Vaast, 2006) also showed that low shade temperatures positively affect bean size, biochemical composition and beverage quality by slowing berry flesh ripening by up to one month. It therefore applies that the night and day coffee temperatures in Ugandan farms are moderated at varying levels by the 88% multitude of randomly planted shade species of varying canopy shade. It is therefore desirable to harvest coffee berries as soon as the pericarp is soft enough to be removed easily during processing as the fruit will continue to respire at a high rate for 2-3 weeks after losing green colour, thus increasing the weight of the fresh pericarp and draining the carbohydrate reserves in the tree.

With the best morphological parameters performance at height 1200 m above sea level and tree age 32-35 years (Table 4.5), and best attainable bean physical characters at 1190 m above sea level at 31

years (Table 54.12) implied that morphological and bean physical characters performed best at 1200 m above sea level with 31-35 year old trees. Even if Thomas, 1947 estimated 10 years as critical age for the survival of a Robusta coffee tree, tree optimum production stage was provided. Thomas, 1947 further noted that the erect type showed superiority in yield and bean size during the first few crops but the "nganda" type would catch up at 4-5 years, a time "erecta" trees start declining and suffer die back from over cropping unless under good management. The highest average screen size 15 beans was produced by trees of 4-10 and 21-50 age range (Table 4.14a) which included vigorous and productive tree age (Table 4.6) with a potentially high carbohydrate competition among beans that can reduce bean size. Also continued crop bearing without adding any soil amendments depletes surrounding soil nutrients subsequently affecting bean size, other physical bean attributes and biochemical quality.

"Nganda" types had the majority of screen size 15 beans with the least density (Figure 4.10a). Naturally, the more spreading "nganda" type or any coffee tree with an open cylindrical shape has many nodes exposed to sunlight causing more flowering and bearing. Cylindrical open trees expose more nodes to sunshine inducing a lot more flowering and bearing. When inadequately nourished, yield may reduce in terms of weight and bean size (Figure 4.10, c). Earlier reports also indicated that carbohydrate competition among berries during bean filling reduced bean size, weight, cup quality and vegetative growth and can be improved by decreasing fruit load (Cannell, 1985; Bertrand et al., 2004; Vaast et al., 2006). Excessive bearing beyond what a tree can sustain also causes foliage reduction, withered branches, and immature beans of reduced bean size result from carbohydrate competition among berries during bean filling (Cannell, 1985, Bertrand et al., 2004, Vaast et al., 2006). When unfavourable conditions or foliage reduction accompany overbearing, physiological stresses such as withered branches and immature beans develop. The small size of

"nganda" leaves though dark (Table 4.3) may trap less sunlight. According to Vaast et al., 2006, heavy fruit bearing traditional coffee varieties with a low leaf to fruit ratios tend to have strong biannual production and quality patterns. The moderated micro climate that results from wide canopy and plant waste may not suffice enough nutrient elements to sustain crop yield.

The large and heavier beans attained by commercial types at farms and hybrid types at Kituza (Table 4.8; Figure 4.4a, b) were the result of selection. The Kituza hybrids were the most recent development, therefore had significantly fewer screen size 15 beans whereas the commercial types had more screen size 15 and above (Table 4.15). The characteristic productive, vigorous, long leaves and internodes, many fruits, few stems and generally young trees for commercial types (Table 4.3) seemed to favour the production of larger bean size by way of adequate nutrient supply. The commercial and hybrid types at farmer fields were estimated at 12 years. Unlike hybrid types that had short internodes and few fruits, trees with long and broad leaves had significantly many fruits (Table 4.3). Seed density can be influenced at bean full formation stage in that the cherry pulp grows as the endosperm absorbs over 70% of the photosynthetic plant products (De Castro et al., 2008). Results also indicated that clay loam soil produced significantly less dense green beans with a high roast volume increase (Table 4.9 b, c). Wrigley, 1988 indicated Ugandan soils had less influence on the distribution of Robusta growing than climate. Coffee beans from sandy loam soil texture were dense as compared to those from clay loam and loam soil texture possibly because the latter had improved aeration and nutrient element access.

CHAPTER FIVE : DIVERSITY OF UGANDAN *Coffea canephora* BIOCHEMICAL COMPOUNDS AS MEASURED BY NEAR INFRA RED SPECTROSCOPY

5.1 Introduction

Biochemical compounds that determine the coffee quality cup arise from precursors in green beans (Thomas, 1935; Davrieux et al., 2003). Flavour and aroma of roasted coffee result from the interaction of many different biochemical compounds such as sucrose, trigonelline, chlorogenic acids (CGA), caffeine and fat. High sucrose and high trigonelline contents are associated with better aroma. Increased concentrations of chlorogenic acids (CGA) and caffeine contents increase bitterness. Fat content helps fix flavour compounds formed during roasting (Davrieux et al., 2003; Charrier et al., 1985). The presence of these compounds helps to discriminate between the different coffee types making them key factors in the determination of organoleptic cup quality. Attributing any of the over 800 aromatic compounds present in roasted coffee to any flavour is not practical. Instead indirect predictors such as chemical compounds (sugars, lipids, proteins, chlorogenic acids, and methylxanthines) through the traditional wet chemistry method and indirect methods such as Near Infrared spectra (Bertrand et al., 2005b) are recommended. Beverage quality for Robusta coffee could be considered as a guiding constraint for selection alongside other major production constraint instead of the traditional method where quality was considered at the end of the cycle (Charrier et al., 1988; Leroy et al., 2006).

The *Coffea* genus includes more than one hundred different species between which a large variation in terms of chemical composition is observed (Clifford, 1985) with *C. canephora* being less aromatic and richer in caffeine than Arabica coffee. Comparison of biochemical variability between cultivated and wild coffee genotypes revealed more variability among cultivated genotypes than wild genotypes with no link between the biochemical groups and agro morphological groups

(Dussert et al., 2004). Montagnon et al., 1998 reported different levels of narrow-sense heritability for Robusta green bean biochemical compounds being as follows; sucrose content (0.11), fat content (0.74), trigonelline (0.39), caffeine (0.80), chlorogenic acid (0.36) and bean weight (0.73).

Other than the genetic diversity of wild coffee and coffee wilt disease phenotypic variability studies (Musoli et al., 2006; 2013), investigation on Ugandan Robusta coffee biochemical compound variability was initiated. Leroy et al., 2006 evaluation 20 *Coffea* species that included Uganda genotypes and revealed Single Nucleotide Polymorphism (SNPs) on some specific genes implicated in sucrose and diterpene metabolisms were influenced by natural and artificial selection and best presumed genes. High nucleotide diversity within protein nucleotide domain or 3'-untranslated region (3'UTR) for three genes that encode enzyme metabolic pathway for KO Kaurene Oxydase, KS Kaurene Synthase and CPS Ent-copalyl diphosphate synthase were revealed in 48 Ugandan erecta, nganda, and cultivated genotypes (Durand et al., 2006). Aluka et al., 2006 found Ugandan Robusta coffee had good cup with some qualities comparable with Arabicas. It is there of paramount importance that characteristics of cultivated and traded Robusta coffee green bean biochemical compounds be understood because of their direct influence on cup quality.

There are modern faster and more reliable techniques used to complement traditional breeding methods of identifying cultivars with superior quality traits that establish presence or absence of a particular substance or elements and its concentration (Davrieux et al., 2003; Downey et al., 1997). Kostiainen et al., 2003 assessed limitations and advantages of each chemical analytical method as follows: Thin Layer Chromatography (TLC) and Flame Ionization Detection (FID) are easy to learn and use but have lower accuracy, inconsistent, sensitive to humidity and the instrument is costly. Atmospheric Pressure Chemical Ionization (APCI), Mass Spectrometry (MS) and Evaporative Light Scattering Detector (ELSD) techniques are less sensitive for substances with

many double bonds such fatty acids. High costs of interpreting Mass Spectrometric Detector (MSD) spectra deter its use. Ultra Violet (UV) analysis does not quantify saturates. A combination of Liquid Chromatography (LC) and Gas chromatography (GC) provides detailed reliable peaks in a short time and high reproducibility. Enzymatic methods have reproducibility and complexity concerns. Titration of fatty acids over estimates the acidity of samples. Gel Permeation Chromatography (GPC) which is similar to HPLC has good reproducibility of standard deviation at different rates of conversion, analyzes classes of compounds but does not separate individual compounds.

Utilization of High Performance Liquid Chromatography (HPLC) technique in coffee chemistry and biochemistry is useful in isolating natural products and predicting physical properties and is not affected by volatile or stable samples (Davrieux et al., 2003). Although costly in terms of chemicals and analysis time, HPLC machine is highly sensitive with a high-resolution power that can used to verify the quality of materials, improve process yields, evaluate product composition and stability, monitor degradation and calibrate analytical instruments such as Near Infra Red spectroscopy (Downey et al., 1997; Davrieux et al., 2005; Huck et al., 2005).

Meanwhile, Near Infra Red spectroscopy (NIRS) measures covalent chemical bonds made up of atoms like carbon, oxygen, hydrogen, and nitrogen (C-H; O-H and N-H) using infrared (IF) light absorption (wavelength and intensity) with an infrared spectrophotometer (Brouwer, 2003). Near infra red range of 14000-4000^{o}cm^{-1} (2.5-0.8^{o}µm or 800 to 2500 nm) has high energy to penetrate the sample exciting overtone or harmonic vibrations enabling the measurement of large samples in each scan. Ultraviolet-visible is another common electromagnetic band used in analytical applications.

In this chapter, near-infrared spectroscopy (NIRS) was used because it had been proposed as a fast and nondestructive method for predicting and quantifying biochemical composition and physical properties in complex compositions such as agricultural, horticultural and food products (Downey et al., 1994; 1997; Bertrand et al., 2005b). In coffee, NIRS has been used successfully to predict and quantify the biochemical content of green beans as well as identify and authenticate products according to fingerprint (Downey et al., 1994; Davrieux et al., 2005; Bertrand et al., 2005b; Huck et al., 2005). Drug metabolism studies also use spectroscopy techniques (Kostiainen et al., 2003). NIR range permits use of a spectrometer without changing instrument settings for similar compounds and can monitor quality as well as determine blend levels (Downey et al., 1997). It is only if significant deviations beyond experimental error are indicated by NIRS analyses would a detailed investigation by more expensive, complex and time consuming analyses, for example by High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) be warranted to determine the exact cause of the problem. However at low concentrations, Near Infra Spectroscopy technique cannot fully quantify substances.

Ugandan Robusta coffee has been described as mild by the international market (UCTF 2008; 2009). It is there of paramount importance that the green bean biochemical compounds of cultivated Robusta coffee that contribute to the described market characteristics are understood. Typically the “erecta” and “nganda” are the two main Robusta coffee varietal types grown in Uganda while improved research varieties are still gaining ground and occur in small amounts. In farmer fields, these coffee types are still found in mixtures and vary in physiology and inherent characteristic. The climatic conditions, soils, crop husbandry, harvest, post harvest and storage condition tremendously vary. Most Ugandan cultivated Robusta coffee is still dry processed. The present study therefore aimed at characterizing multi-locational cultivated *C. canephora* green bean

biochemical compounds diversity using molecular markers and NIR infra red spectroscopy fingerprint diversity. Characterization of coffee green bean biochemical compounds, being the first of its kind in the Ugandan coffee research will form a basis for future research on quality, initiate standard calibration for predicting coffee biochemical compounds and fingerprints in addition to building up Robusta coffee green bean biochemical data base.

5.2 Materials and Methods

5.2.1 Pre testing green bean biochemical compound diversity and verifying the precision of estimating with HPLC and NIRS

A total of 114 accessions planted in year 2004 and 135 accessions of year 2005 had their leaves assessed for genetic diversity using SSR molecular markers and the green bean evaluated for biochemical compounds using NIRS technique (Table 4.1). To verify the precision of the technique, 16 genotypes from year 2005 NIRS evaluation had their caffeine content assessed by HPLC method. To get a broader understanding of the green bean biochemical composition of cultivated Robusta coffee in Uganda, more locations and number of samples were recommended.

5.2.2 Up scaled collection of farmer Robusta coffee genotypes

Out of the 476 tagged Robusta coffee trees whose morphological characters had been evaluated (as given in Chapter 4), 454 genotypes were evaluated using NIRS technique at the National Crops Resources Research Institute (NaCRRI). Maximum moisture content of 12% was maintained for all clean green beans samples. In absence of a thermostat dehydrating chamber, samples that had a moisture content of above 12% were put in a silica dehydration chamber that reduced the bean moisture content to between 8.0 -12%. About 20 grams of each green bean sample was kept in -20^{o}C freezer for at least a day to ease bean grinding by freezing bean fat. Chilled green beans and the motorized hand grinder were rapidly frozen with liquid nitrogen (-197^{o}C) prior to grinding and

the exercise was repeated three times for each sample. The ground sample was sieved using 1mm sieve mesh. The grinder, sieve and work place were cleaned and sterilized using alcohol of over 95% concentration. An estimated average of 3.0 grams homogenized sample powder was randomly sampled and compactly packed in thoroughly cleaned and alcohol sterilized crystal cells with special lenses. Spectrometer Nirsystem 6500 Foss-Perstorp machine and Software ISI NIRS 2 version 4.11 (Infra Soft International) were used to analyze samples in diffuse reflectance from 400 nm to 2,500 nm (2 nm steps) (Davrieux et al., 2003).

5.2.3 Spectra collection

A beam of infrared light radiation from automated source within the machine was produced and split into two separate beams that passed through the sample and the reference pure sample in a solvent. The quantity of photons absorbed (same frequency) followed Beer- Lambert's Law; $A_\lambda = \varepsilon Cl$ [(absorbance A=log (1/R) = log (1/T) where R; radiation reflected & T; radiation transmitted]. A photon is a distinct discrete (smallest) visible light particle or quantum of the electromagnetic field and basic unit of light which has zero mass, no electric charge with an indefinite long lifetime defined as $E = h\nu$ (where energy (E) is a variable, h is Plank constant and ν the frequency or wavelength). Transmitted light revealed how much energy was absorbed at each wavelength (distance between two successive peaks or troughs of light). Analysis of absorption characteristics revealed details about the molecular structure of the sample. The frequency at which the molecule rotated or vibrated corresponded to discrete energy levels or a particular bond type and mass of atoms on either side. When the two beams of light were reflected back to a detector they passed through a splitter which quickly alternated beams entering the detector. In so doing IR light scanned the sample and had its spectrum collected in diffuse reflectance from 400nm to 2,500nm (2nm steps). The reflected (different frequency) and transmitted radiation gave an absorbance

measurement proportional to the number of molecules encountered which was proportional to the concentration of the constituent (Davrieux et al., 2003). A reference sample prevented fluctuations from affecting the data and allowed the effects of the solvent to be cancelled.

The energy absorption as a function of wavelength or commonly frequency (number of times occurrence per unit time) is referred to as absorption spectrum. Absorption spectrum is characteristic for a particular compound, and does not change with varying concentration hence is the chemical "fingerprint" of the compound. To minimize effects of water, average spectra of different samples were expressed as the second derivative of log (1/R) calculated as a difference between two points (where R^2=coefficient of multiple determination) versus wavelengths to enable characterize and measure major absorption bands (C-O; C-C; C-H) that correspond to the different bio chemicals. Predictive models based on NIRS were used to determine the biochemical content of dry matter, caffeine, chlorogenic acids (CGA), trigonelline, sucrose and fats in green beans. The variables used to determine the structure and concentration of the compounds were shape of the spectrum, the optical path length, and amount of radiation absorbed and absorption coefficient of the reference source (Davrieux et al., 2003).

5.2.4 Data analysis

Data analysis was done as described in Chapter 3, sections 3.2.6 and in Chapter 4 sections 4.2.5. Two way non parametric analysis of variance using Bray Curtis distance measure with 9999 permutations determined significant differences among organoleptic cup groups. Predictive models based on NIRS were used to determine the chemical content of coffee beans. The global NIRS spectrum compared Robusta coffee green bean biochemical compounds from Uganda with those at the CIRAD data base.

5.3 Results

Caffeine estimates obtained from NIRS and HPLC techniques were found insignificantly different **(**Figure 5.1). Differences between NIR and HPLC values were all less than 0.5.

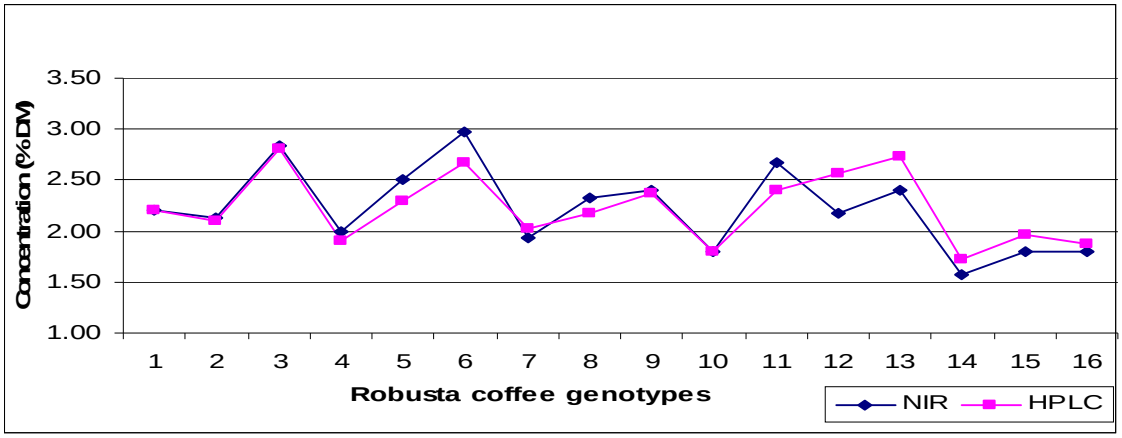


Figure 5.1: Comparison of caffeine values derived from HPLC and NIRS techniques

Different samples absorbed different light intensities at varying wavelengths (Figure 5.2. The amplitude and wavelengths also varied.

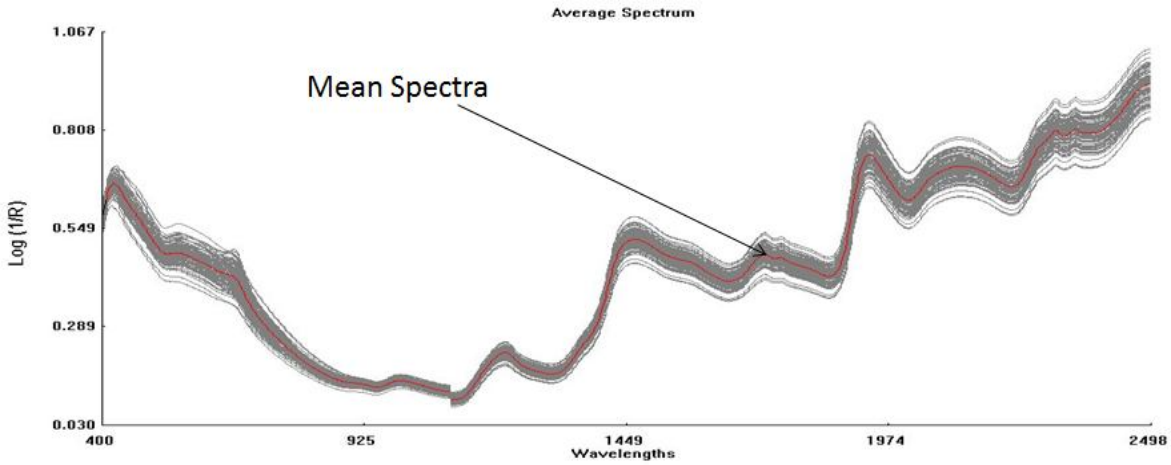


Figure 5.2: Robusta coffee Near Infra Red spectroscopy analysis spectra plot of absorbed light at a corresponding wavelength (red line shown by the arrow is the mean spectrum)

It was apparent that the Ugandan Robusta coffee green bean biochemical compound diversity was different from that in CIRAD data base as shown in Figure 5.3. The difference was more evident when Ugandan samples were few and decreased when evaluated genotypes increased. There were more some genotypes from CIRAD related with clearer

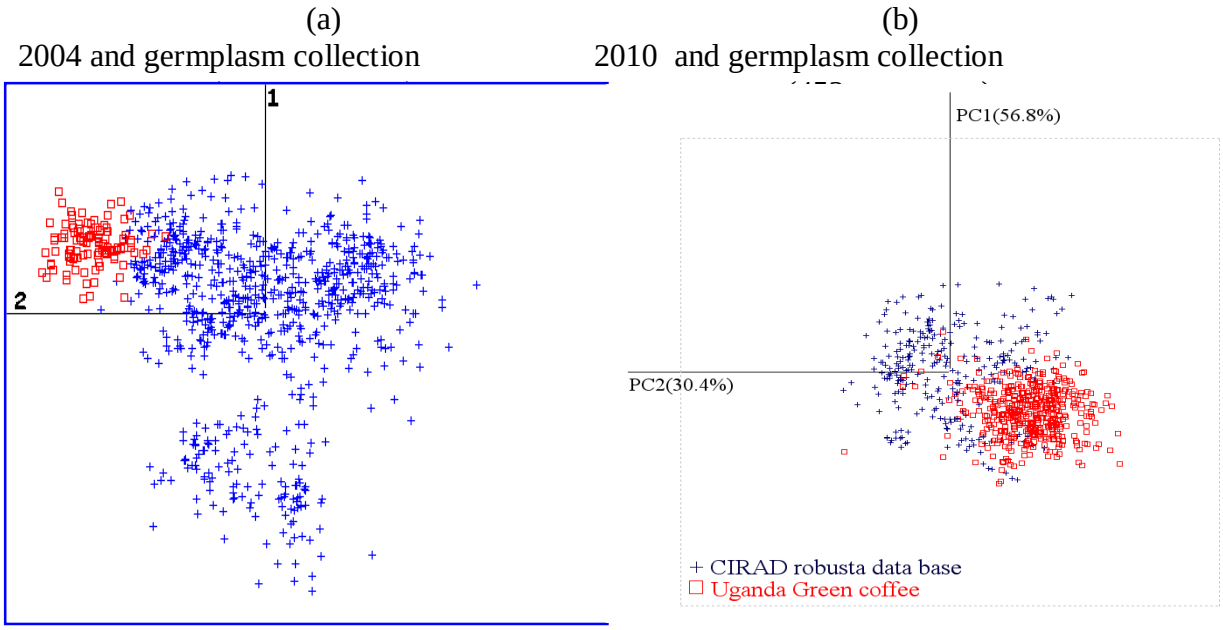


Figure 5.3: Two year principal component plot for Ugandan and CIRAD Robusta coffee green bean biochemical compound data base

Key to Figure 6.3: PC1=63.9%; PC2=12.5%; PC3=5.56%

Analysis of DNA for same genotypes evaluated for green bean biochemical compounds using Near Infra Red spectroscopy with Darwin Neighbour Joining tree revealed three major genetic diversity groups (Figure 5.4). One group clearly subdivided into two. One group was dominated by genotypes from Hoima with a few others from the neighboring district of Kabarole and the far distant district of Jinja. Another group had mixed collections from Kamwenge, Mubende, Kayunga,

Rakai and hybrid types from Kawanda germplasm collection (UH) that were far apart. One sub group was dominated by collections from Kibale while the other sub group was dominated by "erecta" types (UE) from Kawanda germplasm collection. Collections from Kyenjojo were predominant in the Kibale sub group than "erecta" sub group while the Kamuli collections were more in "erecta" sub group than Kibale sub group.

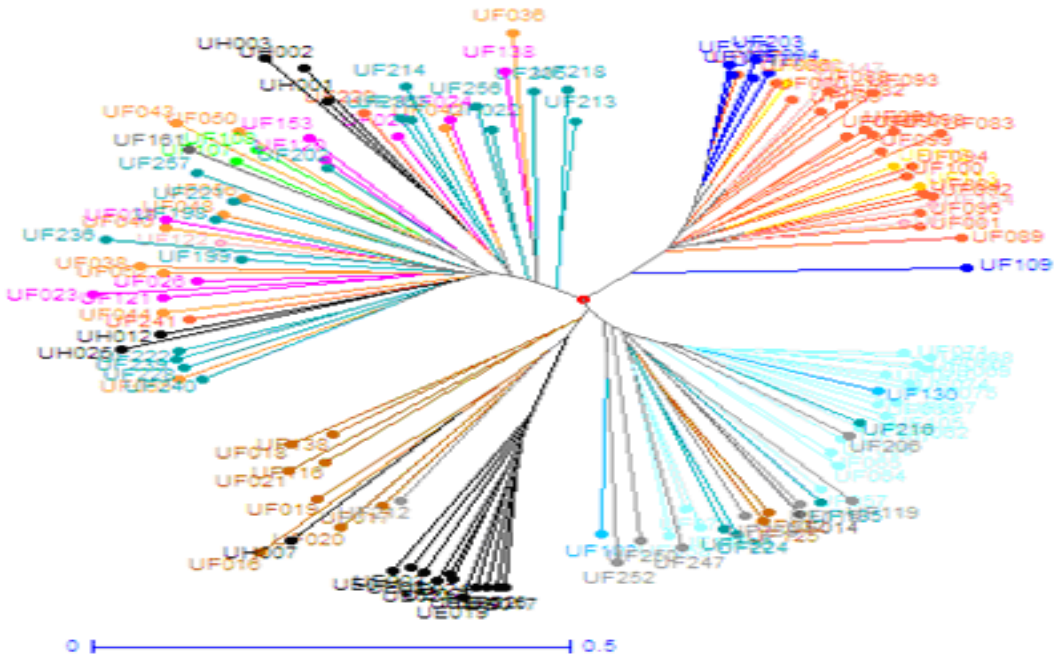


Figure 5.4: Neighbour Joining tree for 135 Ugandan Robusta coffee germplasm DNA analyzed with 18 SSR markers. Legend: UF=Ugandan; UH=Ugandan hybrid types; UE=Ugandan "erecta" types, red=Hoima; deep blue=Kabarole; brown=Kamuli; pink=Kayunga; blue=Kamwenge; light blue=Kibale; orange=Mubende; grey=Kyenjojo; green=Rakai; yellow=Jinja

There were genetic variations in the Ugandan Robusta coffee caffeine concentrations (Figure 5.5). Three major genetic diversity groups for caffeine were formed from collections derived from different locations.

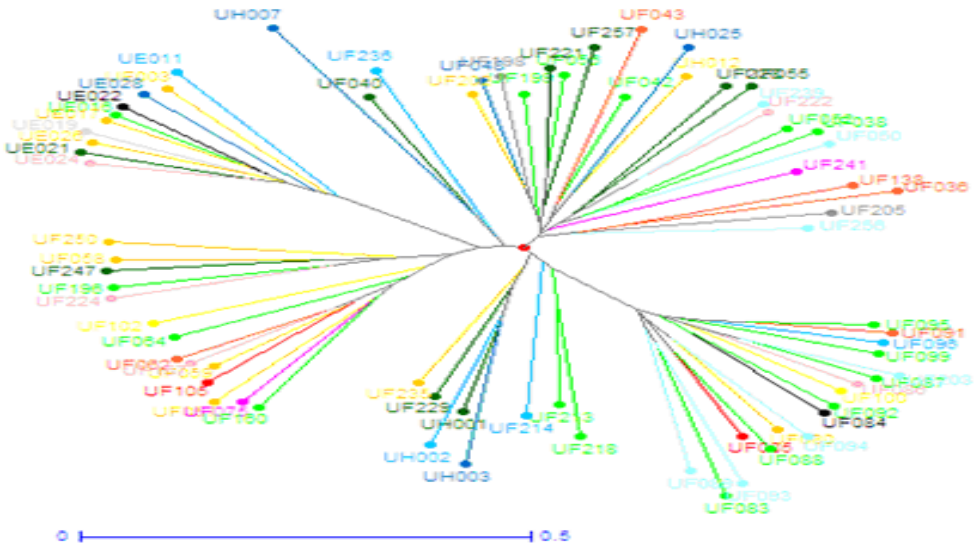


Figure 5.5: Darwin Neighbour Joining tree for caffeine concentrations in Robusta coffee collections. Key: Blue=Kamwenge; black=hybrids from germplasm collection; orange=Mubende; pink=Kayunga; green=Rakai

Cultivated Roobusta coffee biochemical compound variability ranged from 0.37% in trigonelline to 5.7 % in dry matter (Table 5.1).

Table 5.1: Robusta coffee green bean biochemical compound content variability for 452 genotypes (concentration in % of dry matter)

	Dry matter (% dm)	Caffeine (% dm)	CGA (% dm)	Trigonelline (% dm)	Sucrose (% dm)	Fat (% dm)
Minimum	85.82	1.41	10.88	0.66	2.48	10.44
Maximum	91.52	3.29	15.64	1.03	7.34	15.94
Value range	5.7	1.88	4.76	0.37	4.86	5.0
Mean/ s.e	88.26 ±0.04	2.33 ±0.02	12.92 ±0.03	0.79 ±0.004	5.19 ±0.04	13.78 ±0.04

Key to Table 5.1: CGA=chlorogenic acid; s.e=standard error; % dm=percentage dry matter

Table 5.2 is a comparison of Ugandan Robusta coffee green bean biochemical compounds with that reported by other authors. The Ugandan Robusta coffee caffeine content range was 1.41- 5.0% dm

with an average of 2.33% dm whereas Ky et al., 2001a got of 1.0-5.0% dm and Wintgens, 2004 obtained 1.50-2.8% dm with an average of 2.2% dm. Ugandan genotypes had had excessive chlorogenic acid (CGA) content (10.88-15.64% dm) with wide variability as compared to those reported by Wintgens, 2004 (5.34-6.41% dm). Ugandan value range content for trigonelline was 0.66-1.03% dm while Ky et al., 2001a had 0.75-1.24% dm) and values for Wintgens, 2004 were 0.60-1.70% dm. Sucrose concentration in Uganda was 2.48-7.34% dm whereas Ky et al., 2001a had 4.05-7.05 and Wintgens, 2004 rating was 5.0-7.0% dm. Ugandan sample fat content values were as high as 10.44-15.94% dm while Wintgens, 2004 reported a value range of 7.0-11.0% dm for Robusta and 13.0-17.0% dm for Arabica coffee.

Table 5.2: Comparison of Ugandan Robusta coffee green bean biochemical compound variability with samples reported by other authors

Source	Species	Range of biochemical values (% dry matter)				
		Caffeine % dm	CGA % dm	Trigonelline % dm	Sucrose % dm	Fat % dm
Uganda Robusta	Robusta	1.41-3.29 (Av. 2.33)	10.88-15.64	0.66-1.03	2.48-7.34	10.44-15.94
W. Africa Ky et al., 2001a	Robusta	1.0–5.0	**	0.75–1.24	4.05–7.05	**
Wintgens, 2004	Robusta	1.50-2.8 (Av. 2.2)	5.34-6.41	0.60-1.70	5.0-7.0	7.0-11.0
	Arabica	0.70-2.2 (Av. 1.4)	4.80-6.14	1.0-1.20	6.0-8.0	13-17

Key to Table 5.2: **=values not provided by authors; R= Robusta; A=Arabica; Av.=average; % dm=Percentage dry matter

Dry matter and trigonelline content significantly increased with altitude as shown by the positive significant correlation of 0.12 and 0.17 (Table 5.3). Older Robusta coffee trees had significantly reduced dry matter and trigonelline with high caffeine content. Increase in dry matter significantly reduced caffeine and chlorogenic acid but had significant high concentration of sucrose. Increased

bean caffeine content was positively and significantly correlated with chlorogenic acid content (r=0.51 correlation coefficient) but was negatively related with trigonelline, sucrose and fat contents (-0.43, -0.12, -0.17 correlation coefficients respectively). Beans with significant amounts of sucrose had more dry matter, more chlorogenic acid, high trigonelline but less caffeine content. Genotypes with more fat had significantly less dry matter, caffeine but had more chlorogenic acid, trigonelline and sucrose contents.

Table 5.3: The relationships among Robusta coffee green bean biochemical compounds.

Variables	Altitude m.a.s.l	Tree age (years)	Drymatter (% dm)	Caffeine (% dm)	CGA (% dm)	Trigonelline (% dm)	Sucrose (% dm)	Fat (% dm)
Altitude	1							
Tree age	-0.12*	1						
Dry matter	0.12*	-0.11*	1					
Caffeine	0.03	0.15**	-0.10*	1				
CGA	0.07	0.07	-0.26***	0.51***	1			
Trigonelline	0.17***	-0.16***	0.02	-0.43***	-0.06	1		
Sucrose	0.09	-0.08	0.36***	-0.12**	0.21***	0.21***	1	
Fat	0.09	0.00	-0.08	-0.17***	0.38***	0.37***	0.22***	1

*, **, *** significant at 0.05, 0.008, 0.0005 levels of probability; CGA=chlorogenic acid

Overall effect of altitude and tree age on green bean biochemical compounds was highly significant (p= -9.6E-6) with a Wilk's lambda value of 0.90, variance ratio of 3.84 and degrees of freedom for axis 1 and 2 (12, 888 respectively) (Table 6.4) Independent variable effects of altitude and tree age were highly significant (p=0.003; 0.0007 respectively). Dependent variables that were found significantly different were dry matter (p=0.07), caffeine (p=0.02), trigonelline (p=2.3E-6) and sucrose (0.07). Older Robusta coffee trees had significantly more caffeine and reduced dry matter, trigonelline and sucrose (Table 5.4). Trigonelline content significantly increased with elevation. The Biochemical compound variability explained by altitude and tree age was 0.70-5.2%.

Table 5.4: Effects of altitude and tree age on Robusta green bean biochemical compounds

Regression statistic summary							
Biocpds	Predictor variable	coeff	s.e	v.r	F p	t p	R^2(%)
Dry matter	tree age	-4E-3	2E-3	2.65	*	**	0.7
Caffeine	tree age	2E-3	7E-4	5.58	**	**	2.0
Trigonelline	altitude	6E-5	3E-5	13.35	**	**	5.2
Trigonelline	tree age	-5E-4	1E-4	13.35	**	**	
Sucrose	tree age	-4E-3	2E-3	2.60	*	**	0.7
F Stat; F2, 449							

*, **, *** significant at 0.08, 0.05, 0.007 levels of probability; Key to Table 5.4: coeff=regression coefficient; s.e=standard error; d.f=degrees of freedom; v.r=variance ratio; F.p=Fisher level of probability; t.p=Student t-test probability; Biocpds=biochemical compounds (response variable); R^2(%)=biochemical compound variability explained by predictor variables (tree age and altitude).

Analysis of optimum altitude and tree age for obtaining green bean biochemical compounds that offer the best quality show the best altitude was in the range of 1049-1350 m a s l while the most suitable age ranged from 11-57 years (Table 5.5).

Table 5.5: The Gaussian gradient and tree age for green bean biochemical compounds

	Dry matter	Caffeine	CGA	Trigonelline	Sucrose	Fat
Optimum alt	1199.69 ±147.96	1200.91 ±146.66	1200.03 ±148.71	1201.62 ±150.63	1200.46 ±151.16	1200.19 ±149.53
Optimum age	33.98 ±22.59	34.43 ±22.47	34.07 ±22.51	33.65 ±22.51	33.64 ±22.41	33.97 ±22.55
Maximum value	91.52	3.29	15.64	1.03	7.34	15.94

Key to Table 5.5: CGA=chlorogenic acid

There was a significant effect of soil texture (clay, loam, loam clay, sandy loam, sandy/gravel loam) on caffeine content (Table 5.6). Beans derived from clay soils had significantly reduced chlorogenic acid, trigonelline and fat. Coffee beans from loam soil texture had more sucrose.

Table 5.6: ANOVA of Robusta coffee green bean biochemical compounds with soil texture

ANOVA statistic summary							
Biocpds	R^2	v.r	p v	Soil texture	coefficient	s.e	p v
Caffeine	0.04	4.37	***	ns			
CGA	0.03	4.05	***	clay	-1.03	0.35	***
Trigonelline	0.04	4.20	***	clay	-0.07	0.03	**
Sucrose	0.07	7.78	****	loam	0.70	0.37	*
Fat	0.10	12.59	****	clay	-1.76	0.37	****
F Stat; F4, 447							

*, **, ***, **** significant at 06, 0.05, 0.004, 0.0001 levels of probability; Biocpds=biochemical compounds; R^2=coefficient of multiple determination; v.r=variance ratio; p.v=probability value; coefficient=model coefficient; s.e=standard error; ns=no significant soil texture

In Figure 5.6a, loam soil gave the highest mean caffeine content (2.59% dm) while sandy loam gave the lowest (2.32% dm). Beans from loam soil gave the most sucrose (5.25% dm) while clay soil had the lowest (4.2% dm). Most trigonelline content was derived from loam clay while clay soil produced the least amount (Figure 5.6b).

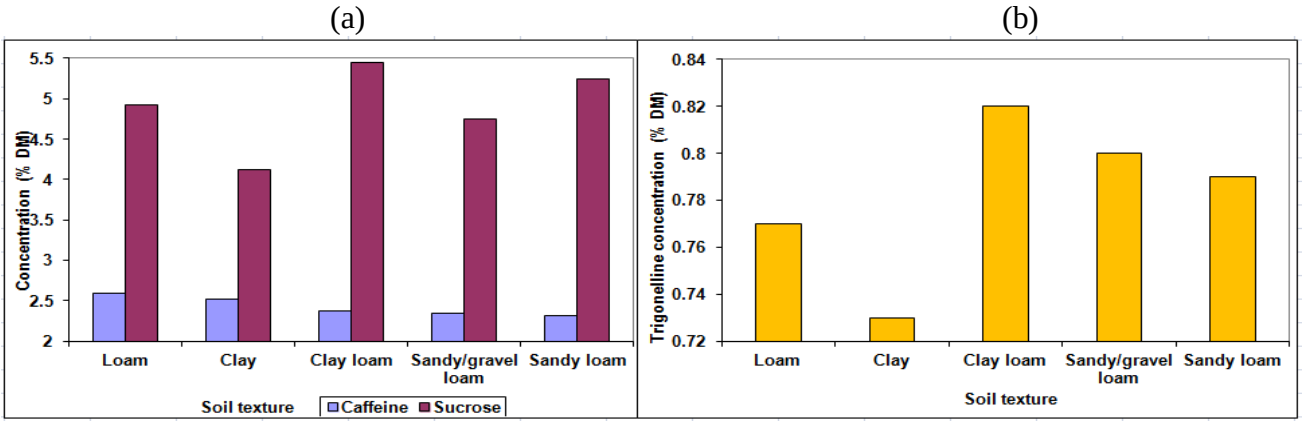


Figure 5.6: Robusta coffee mean bean (a) caffeine, sucrose (b) trigonelline from various soil textures

Beans from sandy/gravel loam gave the most chlorogenic acid and fat content (Figure 5.7). Except for clay soil, the other soil textures gave fat values that exceeded 13.5% dm and chlorogenic acid content of above 12.5% dm.

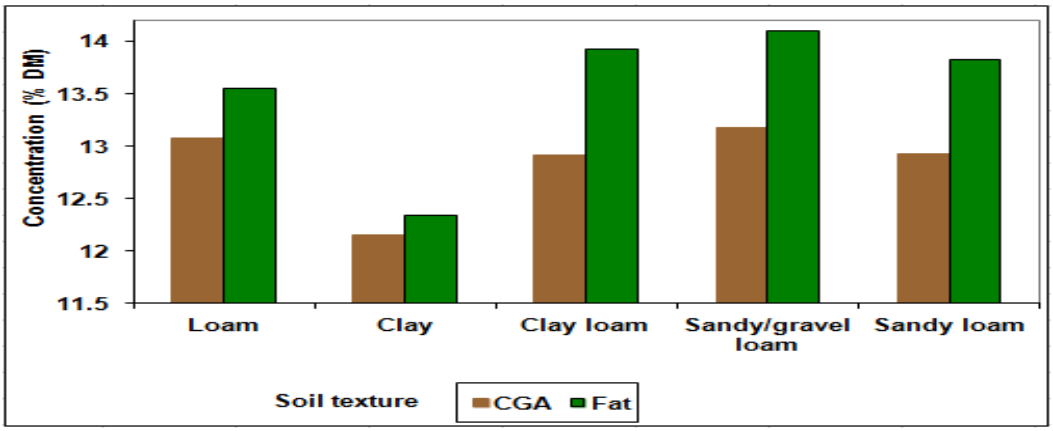


Figure 5.7: Robusta coffee mean bean chlorogenic acid and fat content from various soil textures

In Table 5.7, the hybrids had the most dry matter, least caffeine, chlorogenic acid, and sucrose content. The commercial varieties had also low caffeine, sucrose and fat.

Table 5.7: ANOVA of Robusta coffee biochemical compounds with coffee types

ANOVA statistic summary							
Biocds	R^2	v.r	p v	Coffee types	coefficient	s.e	p v
Dry matter	0.02	2.38	*	hybrids	0.53	0.21	**
Caffeine	0.04	5.86	****	Com, hybrids	-0.16, -0.22	0.06, 0.08	***, **
CGA	0.07	10.75	****	hybrids	-0.71 0.12	0.18	****
Succrose	0.02	3.31	**	com, hybrids	-0.25, -0.67	0.13, 0.2	*, ***
Fat	0.02	2.33	*	com	-0.28	0.13	**
Fstat; F3, 448							

*, **, ***, **** significant at 0.07, 0.05, 0.005, 0.0006 levels of probability

Hybrid coffee types had significantly more dry matter (Figure 5.8a). Commercial and hybrid types had significantly reduced caffeine, chlorogenic acid and sucrose content (Table 5.8a, b). The "nganda" types had the most sucrose while the hybrids had the lowest content (Figure 5.8b). "Erecta" types had the highest chlorogenic acid and caffeine content whereas the hybrids had the lowest quantities.

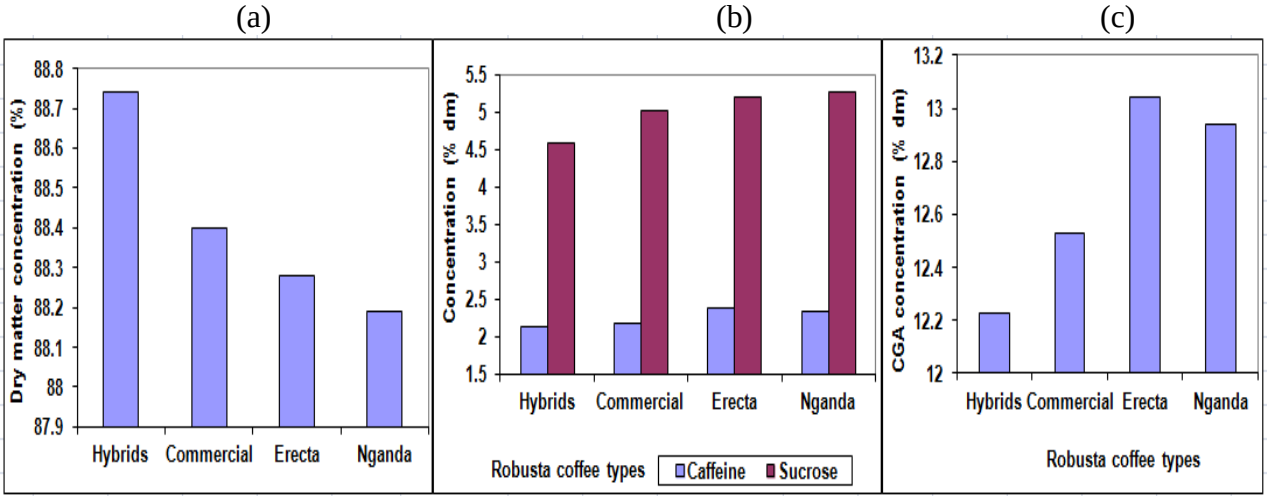


Figure 5.8 (a, b, c): Robusta coffee mean green bean (a) dry matter, (b) caffeine and sucrose (c) chlorogenic acid.

Robusta coffee trees of 31-60 year range had abundant caffeine (Table 5.8). Age range of 4-10 years and 21-70 years had significantly more chlorogenic acid. Trees of 4-30 years had high trigonelline content. Sucrose content was significantly high in tree of age ranges 4-10, 21-40 and 61-70 years. Fat content was more in 21-40 and 61-70 year old trees.

Table 5.8: ANOVA of Robusta coffee biochemical compounds with Robusta coffee tree age range

ANOVA statistic summary							
Biocpds	R^2	v.r	p v	age range	coefficient	s.e	p v
Caffeine	0.09	6.54	****	31-40, 41-50	0.15, 0.28	0.07, 0.07	**, ****
				51-60	0.20	0.07	***
CGA	0.08	5.38	****	4-10, 21-30	0.34, 4.5	0.12, 0.13	***, ***
				31-40, 41-50	0.55, 0.62	0.15, 0.14	****, ****
				51-60, 61-70	0.49, 0.78	0.14, 0.22	***,****
Trigonelline	0.04	2.91	***	4-10, 11-20	0.03, 0.03	0.01, 0.01	***, **
				21-30	0.03	0.01	**
Sucrose	0.04	2.77	***	4-10, 21-30	0.45, 0.47	0.14, 0.15	***, ***
				31-40, 61-70	0.45, 0.58	0.17, 0.24	***, **
Fat	0.03	2.03	**	21-30, 31-40	0.30, 0.35	0.15, 0.17	**
				61-70	0.69	0.24	***
Fstat; F7, 444							

, *, **** significant at 0.05, 0.009, 0.0001 levels of probability

Caffeine gradually increased from 11-20 to 41-50 years when it started declining (Figure 5.9a). Though trigonelline gradually decreased with age until the lowest value at 51-60 year range, there was an abrupt reduction at age 11-20 years (Figure 5.9a) There were two chlorogenic acid reductions at 11-20 years (dominated by commercial and hybrid types) and at 51-60 years with the highest attained content being at 61-70 year range (Figure 5.9b).

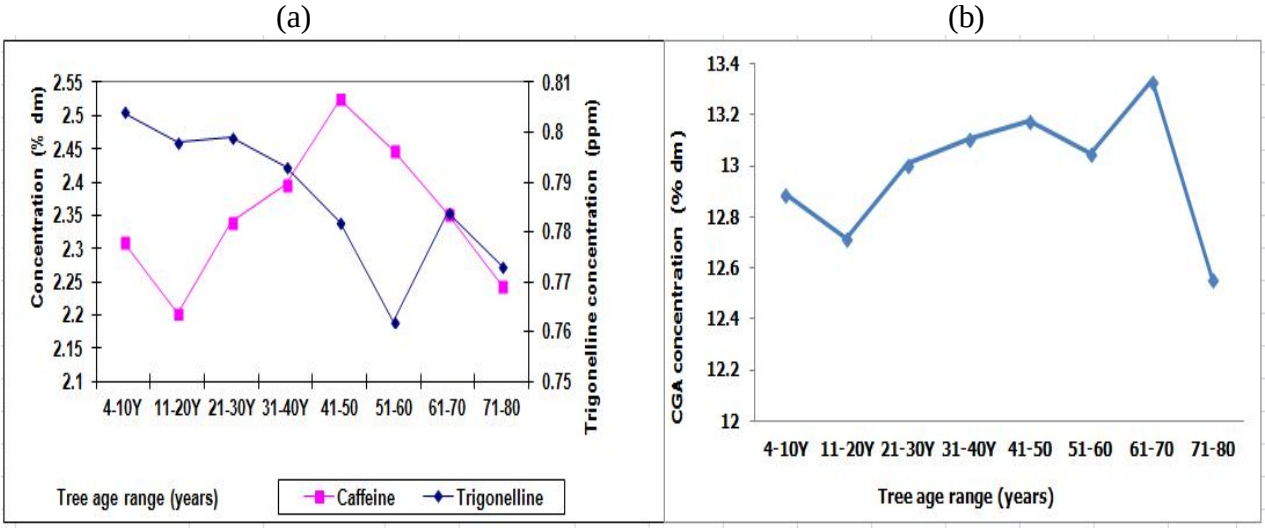


Figure 5.9: Robusta coffee mean green bean caffeine, chlorogenic acid and trigonelline among tree age ranges

In Table 5.9, Robusta coffee green bean biochemical compounds were compared with altitude range. Dry matter was significantly low at altitude range of 1001-1200 m above sea level and 1401-1500 m.a.s l. Caffeine and chlorogenic acid were significantly high at elevation 1301-1500 m above sea level. Meanwhile trigonelline content was significantly low at 1101-1400 m above sea level while sucrose amounts were reduced at 1101-1200 m a.s.l. As for fat content, the scores were significantly low at 1101-1200 m above sea level and significantly increased at 1401-1500 m above sea level.

Table 5.9: ANOVA of Robusta coffee biochemical compounds with altitude range (m above sea level).

ANOVA statistic summary							
Biocpds	R^2	v.r	p v	Alt range	coefficient	s.e	p v
Dry matter	0.07	5.20	****	1001-1100	-0.42	0.20	**
				1401-1500	-0.70	0.24	***
Caffeine	0.03	2.46	**	1301-1400	0.22	0.09	**
				1401-1500	0.17	0.10	***
CGA	0.05	3.77	***	1301-1400	0.41	0.20	**
				1401-1500	0.66	0.21	***
Trigonelline	0.12	9.93	****	1101-1200	-0.06	0.01	****
				1200-1300	-0.03	0.02	**
				1301-1400	-0.05	0.02	***
Sucrose	0.11	9.28	****	1101-1200	-0.44	0.17	***
Fat	0.08	6.05	****	1101-1200	-0.33	0.17	**
				1401-1500	0.46	0.22	**
F Stat; F6.445							

*, **, ***, **** significant at 0.09, 0.05, 0.008, 0.0001 levels of probability

The highest dry matter was realized at elevation 1301-1400 m above sea level with the least values at 1401-1500 m above sea level (Figure 5.10). Trigonelline was more at elevation 1401-1654 while the lowest score was at 1101-1200. At height 1401-1500 m above sea level, chlorogenic acid was high whereas the least values were at elevation 1101-1200 and 1301-1400 m above sea level. Fat values were also high at elevation 1401-1500 m above sea level and low at 1501-1654. m above sea level.

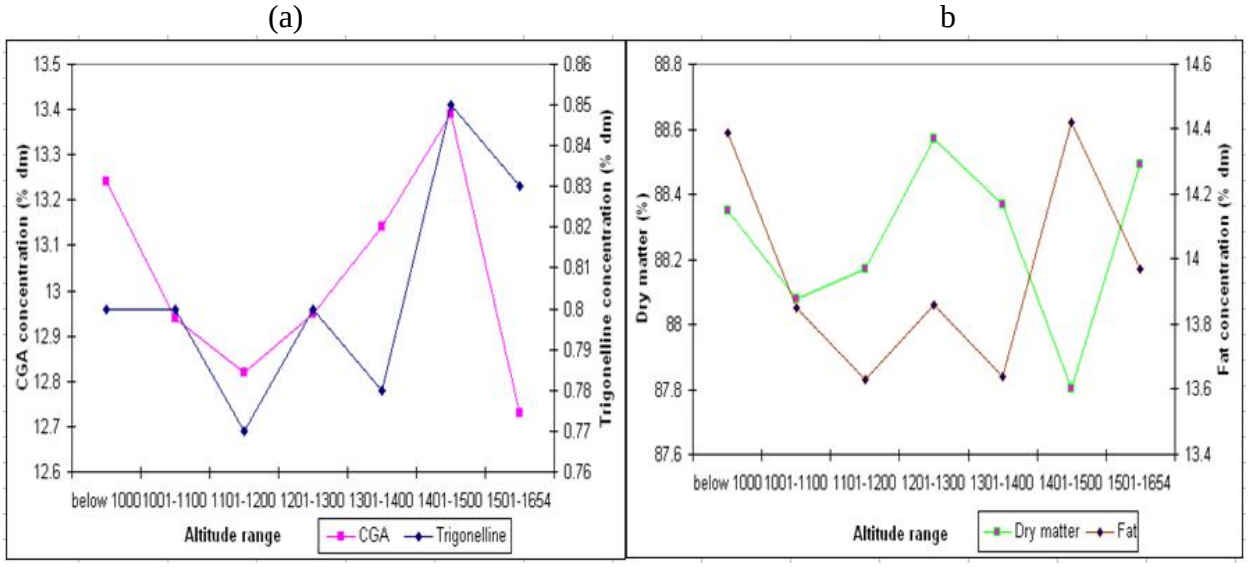


Figure 5.10: Robusta coffee mean green bean dry matter, trigonelline, chlorogenic acid and fat at different altitude ranges (m above sea level).

Out of 24 locations, 15 had significantly low dry matter (Iganga, Bugiri, Bundibudyo, Jinja, Kawanda, Kiboga, Kibale, Kjenjojo, Kalangala, Kamuli, Kayunga, Mbarara, Masindi, Mayuge, Rukungiri) (Table 5.10). Locations of Bushenyi, Bundibudyo, Kawanda, Kamwenge, Kituza, Masindi had low caffeine content. Entebbe, Jinja, Kawanda, Kayunga, Kituza and Mukono had significantly reduced chlorogenic acid. Except for Jinja that was deficient in trigonelline, locations of Bushenyi, Bundibudyo, Entebbe, Jinja, Kabarole, Kiboga, Kamwenge, Masaka, Mbarara, Masindi and Rukungiri had significantly high values. Iganga, Bugiri, Bushenyi, Bundibudyo, Entebbe, Hoima, Jinja, Kawanda, Kabarole, Kiboga, Kibale, Kyenjojo, Kalangala, Kamuli, Kayunga, Kituza, Mukono, Mbarara, Masindi and Mayuge were significantly deficient in sucrose. As for fat content, Iganga, Jinja, Kawanda, KyenjojoJ, Kayunga, Kituza, Mukono were deficient while Entebbe and Mbarara values were significantly high.

Table 5.10: ANOVA of Robusta coffee biochemical compounds with locations

ANOVA statistic summary							
Biocpds	R^2	v.r	p v	locations	coefficient	s.e	p v
DM	0.21	4.91	****	IG, BG	-1.0, -0.97	0.23, 0.25	***, ***
				BU, JJ	-0.60, -0.8	0.26, -0.3	**, ***
				KA, KG	-0.8, 0.7	0.2, 0.25	****, ***
				KJ, KL	-0.63, -0.7	0.2, 0.25	***, ***
				KM, KY	-1.4, -1.1	0.2, 0.2	****, ****
				MR, MS	-1.1, -0.94	0.25, 0.2	****, ***
				MY, RG	-0.85, -0.83	0.27, 0.25	***, ***
Caffeine	0.13	2.67	****	BS, BU	-0.2, -0.33	0.1, 0.11	**, ***
				KA, KW	-0.20, -0.3	0.09, 0.1	**, ****
				KZ, MS	-0.35,-0.3	0.11, 0.12	***, ***
CGA	0.26	6.67	****	EB, JJ	-1.3, -0.86	0.25, 0.2	****, ****
				KA, KY	-0.70, -0.1,	0.18, 0.2	****, **
				KZ, MK	-0.97,-0.8	0.22, 0.22	****, ****
Trigo	0.32	8.66	****	BS, BU	0.08, 0.05	0.02, 0.02	****, ***
				EB, JJ	0.05, -0.04	0.02, 0.02	**, **
				KB, KI	0.06, 0.07	0.02, 0.02	***, ****
				KW, MA	0.07, 0.05	0.02, 0.02	**, ****
				MR, MS	0.01, 0.06	0.02, 0.02	***, ***
				RG	0.01	0.02	****
Sucrose	0.40	12.57	****	IG, BG	-96, -0.97	0.23, 0.21	****, ****
				BS, BU	-0.5, -0.6	0.20, 0.20	**, ***
				EB, HM	-1.7,-1.03	0.25, 0.2	****, ****
				JJ, KA	-1.99, -1.2	0.2, 0.17	****, ****
				KB, KG	-0.70, -1.01	0.20, 0.20	****, ****
				KI, KJ	-0.8,-1.2	0.21, 0.2	****, ****
				KL, KM	-1.2, -1.5	0.21, 0.2	****, ****
				KY, KZ	-0.80, -1.5	0.20, 0.2	****, ****
				MK, MR	-1.0, -0.5	0.21, 0.2	****, **
				MS, MY	-0.66, -1.06	0.24, 0.22	***, ****
Fat	0.26	6.71	***	IG, EB	-0.49, 0.6	0.25, 0.3	**, **
				JJ, KA	-1.30, -0.5	0.2, 0.2	****, **
				KJ, KY	-0.45,-0.5	0.22, 0.22	**, **,
				KZ, MK	-0.57,-1.2	0.2, 0.24	**, ****
				MR	0.5	0.23	**
Fst; F23, 428							

, *, **** significant at 0.05, 0.009, 0.0001 levels of probability

Key to Table 5.10: Refer to Table 4.1

Dry matter content was highest in Entebbe and Rakai while Kamuli and Mbarara had notably the lowest (Figure 5.11a). Sucrose records were high in Kamwenge, Masaka, Rakai, Rukungiri and least in Jinja and Entebbe (Figure 5.11b) .

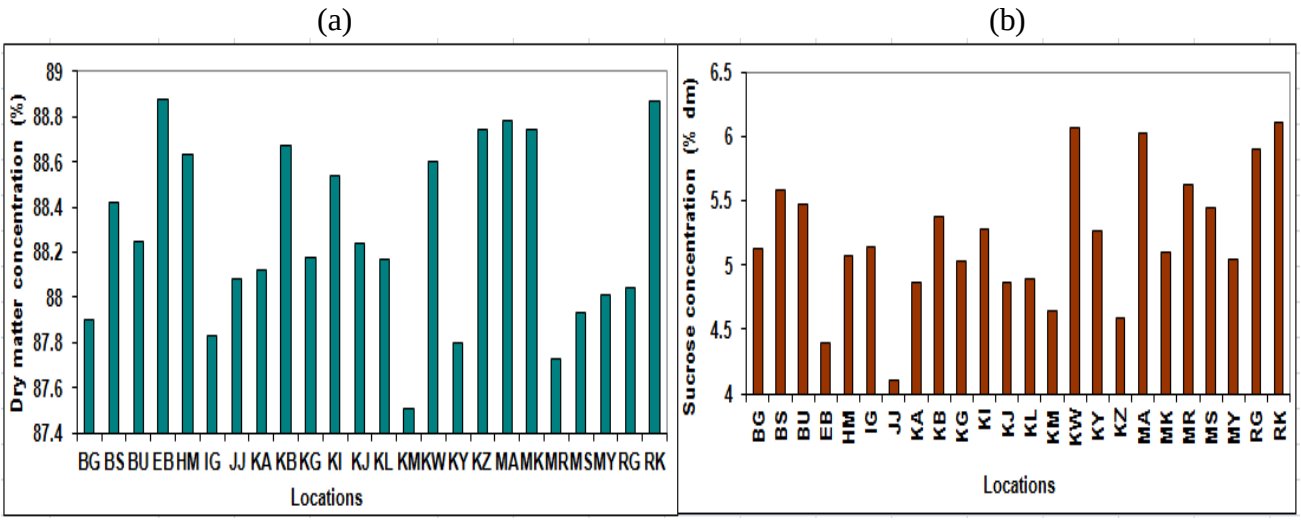


Figure 5.11: Robusta coffee mean green bean (a) dry matter and (b) sucrose at locations

Locations of Jinja, Mayuge Kibale and Kiboga had high caffeine while Kituza, Masindi, Bundibudyo and Kamwenge had the least values (figure 5.12a). Chlorogenic acid was most in Kiboga, Mbarara and Mayuge and least in Entebbe and Kituza hybrids (figure 5.12b). Beans with high fat content were from Entebbe, Mbarara, Masindi while lower values were noted for Jinja and Mukono.

(a) (b)

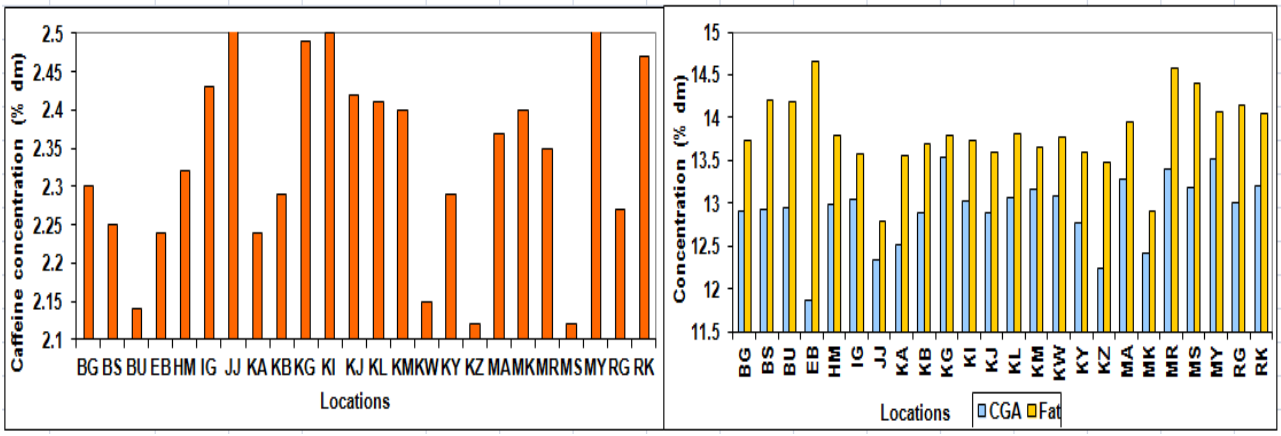


Figure 5.12: Mean green bean (a) caffeine (b) chlorogenic acid and fat from locations

Locations of Rukungiri, Mbarara and Bushenyi had more trigonelline values while Jinja and Mukono had the least (Figure 5.13).

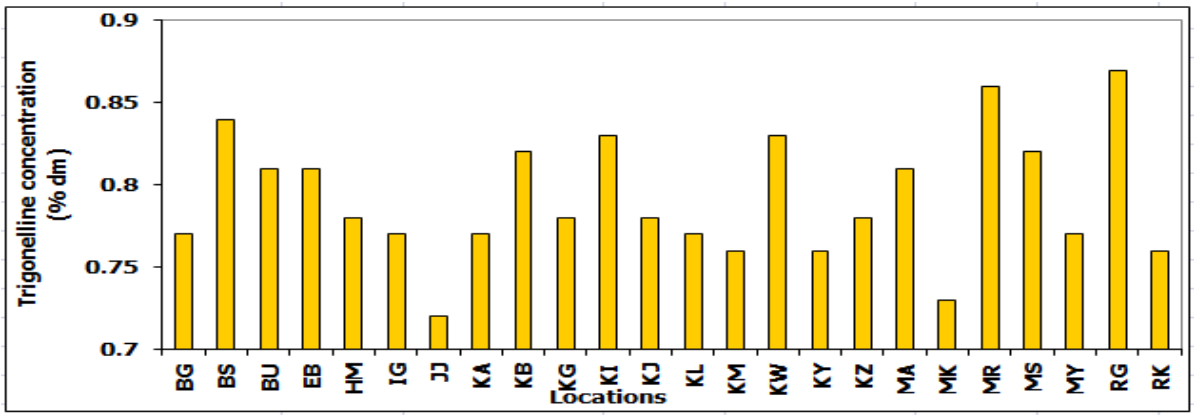


Figure 5.13: Robusta coffee mean green bean trigonelline content at locations

There were 162 genotypes with high amounts of caffeine and trigonelline compounds spread over 24 locations Table 5.11. Phenotypes with high chlorogenic acid and fat content spread in all the 24 sites while beans with more dry matter and sucrose were wide spread in 23 locations. Location attribute minimum values were one genotype with a maximum range of 9 to 18 and mean range of

2.17 to 3.80 genotypes. Beans rich in caffeine had high trigonelline content while those with high chlorogenic acid had the most fat content and the densest beans had more sucrose concentration (Table 5.11).

Table 5.11: Aggregation of 452 genotypes based on highest green bean biochemical compound variance from the principal component analysis

F	Eigen value	Var (%)	Cum %	Variables	Site	Min	Max	Mean	SDEV	Total
F1	3.53	50.43	50.43	caf, trig	24	1	12	6.75	2.69	162
F2	0.98	14.02	64.45	CGA, fat	24	1	18	5.63	3.80	135
F3	0.78	11.16	75.61	dm, suc	23	1	9	3.57	2.17	82

Key to Table 5.11; F=factor; F1=factor 1; Var%=variability percentage; Cum%=cumulative percentage; caf=caffeine; trigo=trigonelline; CGA=chlorogenic acid; dm=dry matter; suc=sucrose

Four partial diversity groups were formed in each quadrant (Figure 5.14). One category had more caffeine while another had more chlorogenic acid. Another accessions group had more dry matter, fat, sucrose and trigonelline. Another lot of accessions had low chlorogenic and fat content. Green bean biochemical compound diversity was dominated by genotypes from Bundibudyo, Bushenyi and Bugiri. Biochemical compound variability in axis 1 was 30.07% and 28.44% in axis 2.

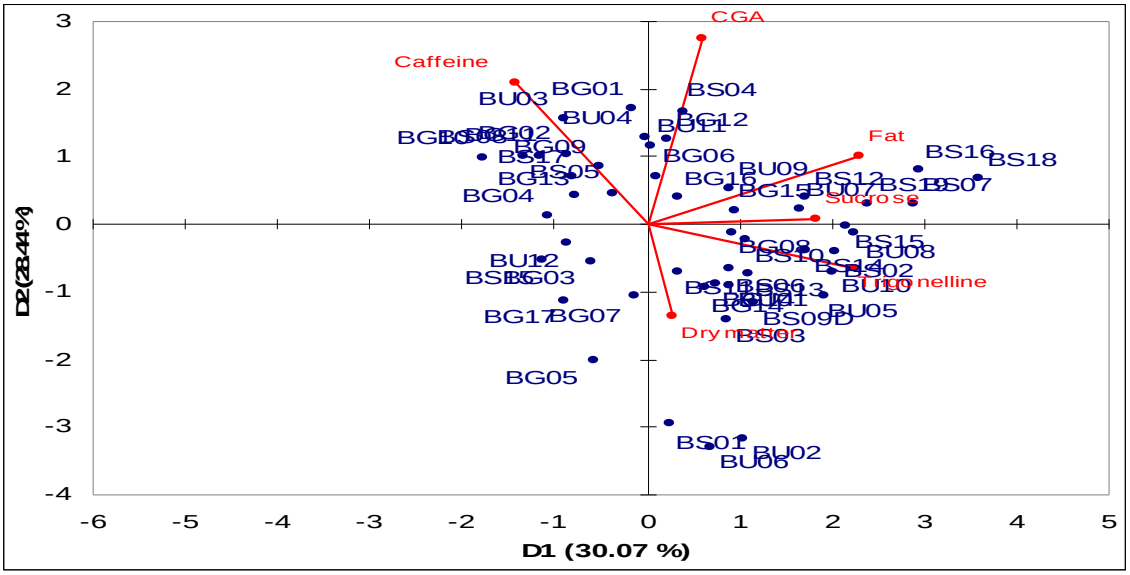
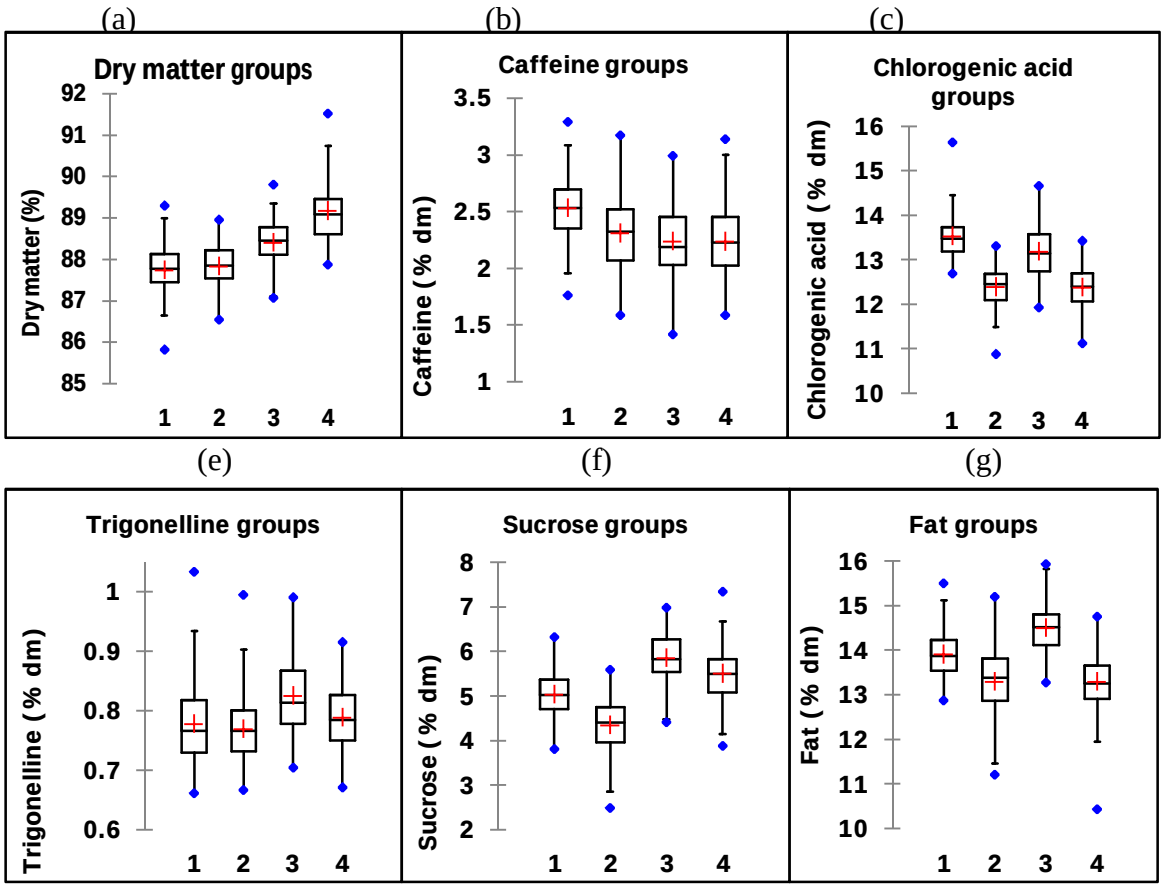


Figure 5.14: Principal component analysis spatial representation of Robusta coffee green bean biochemical compound diversity

Key to Fig 5.14; BG=Bugiri; BS=Bushenyi; BU=Bundibudyo

Caffeine and trigonelline attribute groups had the most variance compared to other groups Figures 5.15 (a-g). Highest and lowest mean, median and interqurtile range dependend on the attribute.



Figures 5.15 (a-g): Descriptions of Robusta coffee green bean biochemical compound diversity groups using box plots

Mahalanobis distances (H) between classes were all higher than 3 and all probability values for Fisher distances were highly significant (<0.0001), indicating that the groups significantly varied in biochemical compound concentrations (Table 5.12). Both Mahalanobis distances and Fisher distances revealed population 2 and 3 were the farthest while 1 and 3 were the nearest with Mahalanobis and Fisher distances of 14.22; 199 and 535.17; 72.51 respectively.

Table 5.12: Estimated Mahalanobis and Fisher distances among Robusta coffee green bean biochemical compound groups as per factorial step discriminant analysis.

Gp	Mahalanobis distances				Fisher distances				p-values for Fisher distances			
	1	2	3	4	1	2	3	4	1	2	3	4
1	0				0				1			
2	7.11	0			99.78	0			<0.0001	1		
3	5.17	14.22	0		72.51	199.53	0		<0.0001	<0.0001	1	
4	11.19	7.80	7.90	0	157.1	109.39	110.90	0	<0.0001	<0.0001	<0.0001	1

The PCA relationships of green bean biochemical compounds with altitude and a plot of K-means analysis groups using a factorial step discriminant analysis was compared in Figure 5.16 (a, b). Group one had more dry matter, less caffeine and chlorogenic acid from relatively younger trees. Group 2 beans were deficient in sucrose, trigonelline and fat with minimal effects of altitude. Group 3 had more sucrose, trigonelline and fat and was influenced by altitude. Group 4 had more caffeine and chlorogenic acid that was positively correlated with aged trees and low dry matter content that had inverse relationship with tree age.

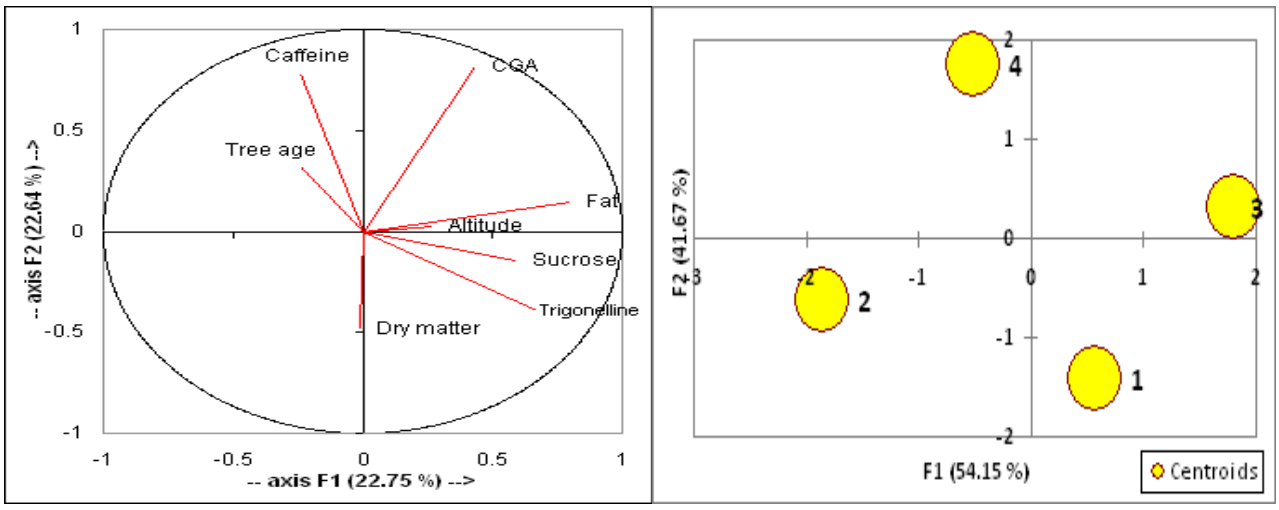


Figure 5.16: (a) Relationships of bean biochemical compounds with altitude, (b) bean biochemical compound groups derived from factorial step discriminant analysis
Key to figure 6.13: X and Y axes represent plot variance.

Estimates of genotype correct placement are shown in (Table 5.13). The chance that genotypes were placed in right groups was about 94%.

Table 5.13: Percentage correct genotype group placement and pair wise distance comparison for Robusta coffee green bean biochemical compounds using a confusion matrix

Confusion matrix						
from \ to	1	2	3	4	Total	% correct
1	120	2	6	0	128	93.75
2	3	95	0	3	101	94.06
3	5	0	113	2	120	94.17
4	1	5	1	96	103	93.20
Total	129	102	120	101	452	93.80

The variance contributed by each attribute to group formation and the group mean averages are shown in Table 5.14. Most variance for group formation was contributed by dry matter (23.27%) followed by sucrose (22.88%) and least by trigonelline (1.74%). Group 1 had more chlorogenic acid and caffeine with the lowest dry matter content and moderate sucrose, fat and trigonelline. Group 2 had the lowest values for dry matter, sucrose, fat, chlorogenic acid and trigonelline with moderate caffeine. Group 3 was described as having the most sucrose, fat, trigonelline with moderate amounts of dry matter, chlorogenic acid and caffeine. Group 4 was explained as having high amounts of dry matter with low values of fat, chlorogenic acid, caffeine and moderate amounts of sucrose and trigonelline.

Table 5.14: Pooled Robusta coffee green bean biochemical compound variance contribution and mean group content derived from Bray-Curtis distance measure

	Variance		Mean group abundance			
	Contribution	Cumulative %	1	2	3	4
Dry matter	0.40	23.27	87.7	87.8	88.4	89.2
Sucrose	0.39	46.15	5.02	4.33	5.85	5.49
Fat	0.38	68.39	13.9	13.3	14.5	13.3
Chlorogenic acid	0.36	89.2	13.5	12.4	13.2	12.4
Caffeine	0.16	98.26	2.53	2.3	2.23	2.23
Trigonelline	0.03	100	0.78	0.77	0.83	0.79

One way multivariate analysis of similarity (ANOSIM) revealed a mean rank within groups (6224), mean rank between groups (1.19E04), R^2 (0.54) and probability value of {P (same): 0.0001} as found in green bean biochemical group pair wise comparisons using the Bray-Curtis distances (Figure 5.17). From Figure 5.17, between class mean, variance and inter quartile range was higher than within group. Group 1 and 4 had comparably high median, variance and inter quartile range. Group 3 had the least median and inter-quartile range. The variance for group 2 and 3 were comparably low and hence more stable groups.

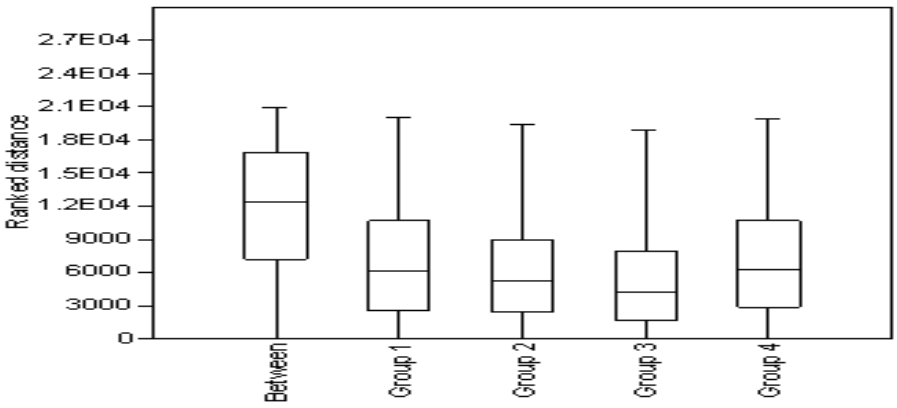


Figure 5.17: Overall description of Robusta coffee green bean biochemical biodiversity using ANOSIM box plots

Table 5.15 compares multivariate biochemical compound groups using a two way non parametric multivariate analysis of variance. There were significant differences among multivariate groups for dry matter and caffeine. Increased dry matter significantly decreased caffeine content.

Table 5.15: Two way non parametric multivariate analysis of variance for the four green bean biochemical compound using Bray Curtis distance measure with 9999 permutations

Source	Sum of sqrs	df	Mean square	F	p
DM	0.033141	6	0.0055235	1.1672	0.0001
Caffeine	0.024494	2	0.012247	2.5881	0.0001
Interaction	-1.6473	12	-0.13727	-29.009	0.047
Residual	2.0396	431	0.0047322		
Total	0.44991	451			

5.4 Discussion

Near Infra Red spectroscopy (NIRS) and High Performance Liquid Chromatography (HPLC) biochemical analyses methods provided comparable results (Figure 5.1). Discriminating cultivar biochemical contents using titration methods is more time consuming and expensive than that based on spectral data (Bertrand et al., 2005b). Furthermore, NIRS technique analyzes many samples in a shorter time and group species according to biochemical finger prints (Davrieux et al., 2003). According to Huck et al., 2005 the minimal detectable caffeine content termed as lower detection limit (LOD) using NIRS technique was estimated at 0.05 g/100 g compared to 0.244–0.60 ng/100 g in Liquid Chromatography. As for the results found here, NIRS offers an alternative to chromatography methods for research and coffee trade because of it is cost effectiveness.

Simple sequence repeat markers and Near Infra Red spectroscopy technique revealed a high degree of green bean biochemical variability among Ugandan Robusta coffee genotypes (Tables 5.1; 5.2). These results were different from those in CIRAD data base (Figure 5.3) that showed that Robusta

coffee might have unique gene pools in various centers of origin and evidence that CIRAD had not earlier accessed Ugandan Robusta coffee in their biochemical compound data base. The three major genetic diversity groups with one subdividing into two (Figure 5.4) and the four multivariate green bean biochemical compound groups (Figures 5.14; 5.15a-f; 5.16b; 5.17) imply that a wide range of biochemical compounds exist among Ugandan *C. canephora*. Even if wild forms were not assessed, greater biochemical variability was anticipated among cultivated than in wild genotypes as reported by Dussert et al., 2004.

These findings further confirm that Uganda is within the centre of origin (Musoli et al., 2009) offering the opportunity for selecting bean biochemical compounds of interest. While Leroy et al., (1993) established that Robusta coffee biochemical groups were related to molecular groups, Montagnon et al., 1998 found that genetic factors greatly influenced green bean biochemical composition. It was therefore probable that the three main genetic diversity groups shown in Figures 5.4 and 5.5 offer heritable traits while the four principal component plots (Figure 5.14) and multivariate biochemical compound diversity groups (Table 5.14; Figures 5.15; 5.16) reflected the combined effect of inherent traits and environment. It is also likely that accessions x environment interactions could have contributed to non-discrimination of distinct clusters in certain cases.

To further illustrate that bean biochemical compounds were influenced by genetic factors, dry matter, fat and chlorogenic acid contents with high heritability had variance contribution values of (23.27%), (22.24%) and (20.81%) to multivariate group formation while sucrose known for intermediate heritability contributed 22.88% (Table 5.14) (Leroy et al., 2006; 2008; Tessema et al., 2011). It therefore implied that preliminary selection for beans with desirable biochemical constitution should consider highly heritable traits and genotypes with superior physical bean characters and organoleptic characters. Lack of relationships between the sites and diversity groups

derived here (Figures 5.4; 5.5) may have been due to common selection practices that could have minimized genetic differentiation. The practice of sharing planting material in form of cuttings, volunteer seedlings and seed, use of wild collections and land races could have contributed to absence of differences between growing sites. In addition, high biochemical compound variability could be attributed to the freely crossing nature between wild forms and Robusta land races (Maitland, 1926; Thomas, 1940; Leakey, 1970).

From the multivariate analysis, genotypes that comprised mainly old trees had significantly more caffeine (Table 5.8; Figures 5.14; 5.16), reduced dry matter, fat, trigonelline and sucrose values, indicating that beans from old trees were less dense and likely to produce beans with reduced aroma and flavor with a bitter brew. High caffeine production may have been as a result of physiological stress associated with aging trees or chemical defense against attack or serve as an antiherbivory and allelopathic compound (Uefuji et al., 2003). The results also showed that one of the diversity groups could target for a market niche that requires coffee with limited sucrose, trigonelline, fat chlorogenic acid with moderate dry matter and caffeine (Table 5.16). Another niche may need beans of medium size, with reduced aroma, flavor and bitterness. The third category of genotypes that were influenced by elevation had the highest concentration of fat, sucrose, and trigonelline with moderate dry matter, chlorogenic acid and caffeine (Figure 5.16b). These genotypes gave average weight beans which were aromatic, flavoured and of average bitterness. Coffee beans with more dry matter, low chlorogenic acid, caffeine, fat with moderate sucrose and trigonelline may offer an aromatic and flavoury cup which is less bitter preferred by some markets. Aggregating 452 genotypes based on highest green bean biochemical compound variance from the principal component analysis showed that genotypes with the highest biochemical compound were wide spread in all locations (Table 5.11) with a minimum value of one genotype

and maximum number of 18 genotypes. Although green bean biochemical compound variability was expected among cultivated than wild genotypes (Dussert et al., 2004), the PCA pointed out that genotypes from Bundibudyo, Bushenyi and Bugiri that were distantly located had the most variability (Figure 5.14).

The significant differences among the four multivariate bean biochemical compound groups for dry matter and caffeine (Table 5.14) and the inverse occurrence of dry matter and caffeine (Table 5.3; Figures 5.14; 5.16) in the green beans implies the opportunity to select for dense beans with reduced caffeine content. Montagnon et al., 1998 found that bean weight and biochemical compounds such as fat and caffeine had high heritability values and were good choices of parents to make crosses. Tessema, 2011 also found high heritability expressed in dry matter, ash, protein and fat.

Comparing Ugandan bean biochemical compounds with those in West Africa (Table 5.2) showed that Ugandan coffee had much more fat content (10.44-15.94% dm) chlorogenic acid (10.88-15.64% dm) and sucrose content (2.48-7.34% dm) than those reported by Wintgens, 2004 from West Africa (7.0-11.0% dm; 5.34-6.41% dm; 5.0-7.0% dm respectively). Preliminary studies by Aluka et al., 2006, found Ugandan Robusta coffee cup test was good with some qualities comparable with Arabicas and that fat and chlorogenic acid contents were high. Even if some genotypes had as high caffeine content as 3.29% dm, on average cultivated Ugandan Robusta coffee mean value of 2.33% dm was comparable to that reported by Wintgens, 2004 (2.2% dm). Ugandan trigonelline lower values of 0.66-1.03% dm were comparable to those by Ky et al., 2001a (0.75-1.24% dm) and Wintgens, 2004 (0.60-1.70% dm), but with low maximum values. Chlorogenic acid and caffeine alkaloids contribute to the organoleptic properties of coffee beverage, particularly the bitterness and astringency (Ky et al., 2001a). High sucrose and trigonelline contents produce better aroma while

fat content help fix flavour compounds formed during roasting (Davrieux et al., 2003; Charrier et al., 1985).

Genotypes of “nganda” types had the most sucrose and reduced dry matter (Table 5.7), hence high aroma and flavoured coffee of possibly reduced roasted ground bean volume. Trees of age range 4-10, 21-40 years were productive and vigorous as shown in Table 4.10 whereas trees of 61-70 years less productive. Equally, “nganda” accessions from Kamwenge, Masaka, Rukungiri and Rakai had more sucrose while those from Jinja and Entebbe had the least (Table 5.8; 5.10). According to Geromel et al., 2008, shade sucrose content varied according to genotypes as was the case with *C. arabica* cultivars where ‘Catuai’ had more sucrose while that for the ‘Catimor’ was reduced. Synthesis of reducing sugars and sucrose is also known to increase at fruit ripening (De Castro et al., 2006; Geromel et al., 2006). With over 90% of coffee trees observed to be under tree shade coupled with the wide sucrose range revealed by results (Table 5.2) implied either the shade trees had scanty canopy or the sucrose content was highly influenced by genotype x environmental factors.

Ugandan Robusta coffee had high amounts of chlorogenic acid (10.88-15.64% dm) as compared to Wintgens, 2004 (5.34-6.41% dm) (Table 5.2). Other than the effect of sun grown beans being associated with high sucrose, chlorogenic acid and trigonelline concentrations (Muschler et al., 2001; De Castro et al., 2008), other factors could have contributed to high chlorogenic acid content. For example a gradual increase of chlorogenic acid and caffeine was observed in trees of 11-20 years until 41-50 for caffeine and 61-70 for chlorogenic acid (Table 5.8) possibly because the trees were highly productive at 11-30 (Table 4.9). In this case, tree age and the number of fruits may have varied the biochemical compounds deposited in the bean. In Table 5.9, increase in elevation

also increased dry matter, caffeine, chlorogenic acid and fat content until at altitude 1201-1300 m above sea level for dry matter and 1301-1400 m above sea level for caffeine, chlorogenic acid.

The lowest fat content was realized at 1101-1200 m above sea level and highest at 1401-1500 m above sea level. In Arabica traditional varieties, Bertrand et al., (2006) reported chlorogenic acid and fat concentration increased with elevation but not caffeine content. Arabica coffee, normally grown in highland areas has more trigonelline as compared to Robusta coffee. Ugandan Robusta coffee is cultivated mainly between altitude range 1000 to 1500 m above sea level (Wrigley, 1988), a height which is relatively high as compared to in India where cultivation is favourable at 500-1000 masl. This is possibly the reason why dry matter, caffeine, chlorogenic acid and fat compounds were significantly high in the Ugandan Robusta. Also beans from Kiboga (1136-1373 m above sea level), Mbarara (1383-1478 m above sea level) and Mayuge (1181-1217 m above sea level) had significantly high levels of chlorogenic acid while those from Entebbe (1177 m above sea level) and Kituza (1200 m above sea level) hybrids had the lowest (Table 5.10; Figure 5.12b). Except for Mbarara and some places in Kiboga, the rest of the locations were in the lowland range. The low occurrence of caffeine and chlorogenic acid content in hybrid and commercial types could be aettributed to the selection of physical bean and organoleptic characteristics by research institutions.

Correlations among biochemical compound indicated that caffeine content was positively and significantly correlated with chlorogenic acid content but was negatively correlated with trigonelline, sucrose, fat and dry matter contents (Table 5.3). This relationship would be advantageous especially in situations where selection for the latter four biochemical compounds is favoured as this would lead to reduced caffeine and chlorogenic acid. As found by Campa et al., 2005b the Ugandan samples had more chlorogenic acid than caffeine alkaloid (Table 5.1; 5.2),

indicating that not all the chlorogenic acid was converted to caffeine chlorogenate complex in the seed.

In addition to high altitude, old trees of 31-60 years had significantly more caffeine (Table 5.8). Biosynthesis of caffeine at old age could have been in response to stress or attack as a defense mechanism (Uefuji et al., 2003; Ashihara et al., 2008). Caffeine is involved in chemical defense and allelopathy. Jinja, Mayuge Kibale and Kiboga had high caffeine while Kituza, Masindi, Bundibudyo and Kamwenge had the lowest values (Figure 5.12a). High caffeine content among Jinja collections could have been as a result of older trees being cultivated (Table 4.12) while Kamwenge had younger trees. Since the results here showed high caffeine in 30-60 year old trees (Table 5.8), implies older tree age have more bean caffeine.

Trigonelline content increased significantly with age up to 30 years (Table 5.9) but gradually decreased with increased tree age until 51-60 years when the lowest values were reached (Figure 5.9a); implying younger trees had more trigonelline. The highest recorded mean trigonelline value was at elevation 1401-1654 m above sea level (Figure 5.10a) in the highland areas of Bushenyi, Mbarara and Rukungiri (Figure 5.13). This possibly reflects that trigonelline content increases with altitude. The lowest trigonelline score at were at 1101-1200 m above sea level and in the lower locations of Jinja and Mukono.

The commercial varieties had low caffeine, sucrose and fat (Table 5.7), hence were likely to provide less bitter and stringent cup of low flavor and aroma. Lower altitude areas of 1101-1200 m above sea level (Table 5.9; Figure 5.10b) including Jinja, Mukono, Iganga, Kawanda, Kayunga, Kyenjojo and Kituza (Table 5.10; Figure 5.12b) also had low fat content. Although in Arabica coffee, fat was found to increase with altitude in traditional cultivars (Bertrand et al., 2006), in the Ugandan

situation, both high and lowland areas produced beans with high fat. Better management at the shores of Lake Victoria could have influenced the high fat content found in the Entebbe botanical gardens germplasm (Table 5.10; Figure 5.12b). The difficulties in accessing nutrient elements from clay soil could have contributed to the reduced chlorogenic acid, trigonelline and fat content observed in clay soils (Table 5.6; Figures 5.6; 5.7). Significantly high fat scored by 21-40 and 61-70 year old trees (Table 5.8) were from vigorous and productive trees that were able to synthesize carbohydrate with eventual fat synthesis.

It is also important to point out that the lowest fat content of 10.44% dm in the Ugandan condition was comparable to the highest score of 11.0% dm reported in Wintgens, 2004. In Ugandan conditions, except for the Rift Valley where Robusta is grown at an altitude range of 600-1000 m above sea level, cultivation of Robusta coffee goes beyond 1600 m above sea level. It is also observed that coffee plants from areas nearer the Equator and tropics require higher altitudes compared to those near the Tropic of Cancer in the North or Tropic of Capricorn in the South (DaMatta et al., 2006). The high leaf to fruit ratios at higher than in the lower elevation (Vaast et al., 2006) increased carbohydrate supply to berries and subsequent fat synthesis. The slowed berry flesh ripening experienced at lower temperatures but not encountered at higher elevations, may have allowed for longer and better bean filling (Vaast et al., 2006). Low fruit load may improve bean biochemical composition and organoleptic cup quality (Cannell, 1985, Bertrand et al., 2004; Vaast et al., 2006).

Hybrid types had significantly more dry matter with reduced sucrose, caffeine and chlorogenic acid content (Table 5.7), that probably made them bitter but with a reduced aroma and flavour. In addition, prior research selection criteria that considered high seed weight and size among desirable traits, hybrids were exceptionally vigorous compared to land races (Table 4.4). According to

Bertrand et al., (2006), higher vegetative vigor associated with hybrids gave higher leaf to fruit ratios and better carbohydrate supply to berries, irrespective of elevation. Hybrid types had increased ability to physiologically adjust combined with high heterosis (Bertrand et al., (2005a). At average age of 12 years hybrid trees were vigorous and productive (Table 4.4; Figure 4.8). In 14 sites, there was significantly low dry matter in accessions while 20 had significantly low sucrose (Table 5.10). Dry matter content was significantly higher in the hybrids, green beans from Entebbe, Kituza, Masaka, Mukono and Rakai but notably low in Kamuli and Mbarara. Increase in dry matter with height was significant at 1301-1400 m above sea level (Table 5.9). Dry matter and sucrose were significantly reduced at a lower elevation of 1001-1200 m above sea level and a height of 1401-1500 m above sea level in the hybrids.

CHAPTER SIX: BIOCHEMICAL DIVERSITY OF UGANDAN *Coffea canephora* SENSORY ORGANOLEPTIC CUP ATTRIBUTES

6.1 Introduction

Coffee being the most traded agricultural crop world-wide has several classification systems aimed at facilitating market and value addition (Leroy et al., 2006). For instance coffee consumer acceptability using hedonic organoleptic cup evaluation is done by 60 assessors representing the population (Leroy et al., 2006), while in descriptive organoleptic cup analysis, trained assessors discriminate coffees by describing organoleptic cup profile (Leroy et al., 2006). Grading *C. canephora* with a protocol suited for *C. arabica* misleads as the two species differ in various aspects that include quality, consequently the competitive market advantage for Robusta is reduced.

Some of Robusta coffee beverage inherent inferior cup quality characteristics include deficient good flavour, minimal acidity and pronounced bitterness (Charrier et al., 1985; Wrigley, 1988; Prakash et al., 2005). Assertion that Arabica has more acidity and less caffeine underestimates the fact that many consumers prefer neutral coffees. Ugandan Robusta coffees have low caffeine content (UCTF, 2008; 2009). Traditional dry processing and occasional wet processing have not fully enhanced cultivated Robusta coffee quality potential in Uganda. The excellent characteristic cup appearance, used in blends over the past 15 years as soluble coffees or fillers has not helped to improve the market (UCTF, 2008; 2009). Due to these factors, the general market value for *C. canephora* has persistently remained lower than that of *C. Arabica* in spite of the several economically useful traits endowed in *C. canephora* such as tolerance to disease and pest attacks (Prakash et al., 2005).

In Uganda, national efforts in coffee quality improvement are geared towards training on good crop husbandry practices, harvest and post harvest, processing, and production of special coffee grades for

different markets (UCTF, 2008; 2009). Value addition is encouraged through good crop husbandry, processing and marketing of coffee products. Development of a standardized protocol and quality standards for independent sensory assessment of Robusta from Arabica coffee will help unveil cup traits that are specific to Robusta.

Moschetto et al., 1996 established significant differences between the Guinean and Congolese Robusta coffee genetic diversity groups for aroma, acidity, body and bitterness. Montagnon et al., 1998 indicated biochemical compounds and organoleptic cup traits could be improved without affecting yield. High linear correlation coefficients between preference and factors such as acidity and aroma were obtained from cup tasting samples of different genetic groups, hybrids and commercial clones (Moschetto et al., 1996). Arabusta hybrids between *C. arabica* and *C. canephora* have proven unsuitable due to their lower fertility and unstable yields in lowlands (Capot, 1972; Charmetant et al., 1992). Attempts to promote Arabusta hybrids selected for high quality in Uganda as an alternative to Robusta coffee in medium altitude areas where coffee wilt disease had devastated *C. canephora* were futile because farmers preferred *C. canephora*.

Endevours to improve *C. canephora* quality through interspecific crosses have been done using *C. congensis* with large bean size and a better organoleptic quality (Moschetto et al., 1996). *C. liberica* with less bitter brew and larger beans has been crossed to *C. canephora* followed by backcrossing F1 (*C. canephora* x *C. liberica*) to *C. canephora* (Yapo et al., 2003). *C. pseudozanguebariaem* a caffeine free species with high levels of trigonelline and sucrose was also crossed to *C. canephora* (Barré et al., 1998; Ky et al., 1999; 2000a, b; 2001b). Genes that influence variation in coffee quality, population structure and genetic maps have been developed in the coffee genome for exploitation in quality improvement (Combes et al., 2000; Dufour et al., 2001; Poncet et

al., 2004). Quantitative trait loci (QTL) for chlorogenic acid may be used improve cup quality (Campa et al., 2003).

Since Robusta coffee occurs naturally in Uganda and constitutes 80% of the national coffee grown, profitable production could be based on aggressive quality improvement at genotype, crop husbandry, biogeography and processing levels. Unfortunately, neither the diversity nor physical and flavor profiles in different locations are known. No considerable research to date has been conducted to improve cultivated Robusta organoleptic quality or identify cultivars with superior cup characteristics for conservation or for use in quality improvement.

As coffee trade increasingly gets competitive with increased specific market demands, coffee quality, particularly organoleptic cup test becomes an important marketing criterion. Organoleptic cup in different varieties have been associated to inherent traits (Tessema et al., 2011; Dessalegn et al., 2008) which are diverse in wild accessions and farmer landraces. Moreover, of the 103 accepted *Coffea* species, 70% are threatened with extinction but only a few of them have been studied (Poncet et al., 2007). Furthermore, altitude, rainfall, temperature patterns, latitude, sunshine, luminosity among other climate factors, play important roles during the ripening period (Cannell, 1985; Clifford et al., 1985; Decazy et al., 2003). To strengthen the quality improvement program, the present work therefore aimed at establishing the presence and structure of organoleptic cup variability among Ugandan cultivated *C. canephora* in different locations.

6.2 Materials and Methods

6.2.1 Samples and Green bean preparation prior to cupping

To appreciate the different cultivated Robusta coffee fruit flavours, green as well as roasted beans were smelt. The 206 farm collections that had earlier on been evaluated for their morphological and bean physical characters (Chapter 4, sections 2.2 and 2.4) were assessed for organoleptic cup qualities. The three control samples provided by UCDA had the bean physical characters and organoleptic cup attributes assessed.

An experienced Robusta roaster from Uganda Coffee Development Authority (UCDA) roasted 10 clean green bean samples of 55-120 grams each using PROBAT roaster a day before the cupping was done. A maximum of 10 samples per cupping session was intended to minimize possible accumulation of caffeine in the mouth that adversely alters cupping ability. Roasting temperatures were from 150^{o}C to slightly above 200^{o}C. The attained bean color was medium to medium dark roast and the roast time was recorded in minutes and converted to seconds per gram. Clarke et al., 1985 estimated the moisture content of medium to medium dark roasted beans at 7% dry matter subject to atmospheric humidity. The roasted coffee was left to cool up to room temperature and stored overnight in a cool dry place free from other odours and air flow to minimize risk of contamination until cupping. All roasted samples were left to cool for at least 12 hours before the weights and volume of roasted beans were taken. The silver skin was removed by rubbing softly and eventually by use of a motorized blowing machine.

Three separate measurements of 14.0 grams from the same roasted sample were ground to medium size using a motorized laboratory grinding machine and powder kept in three different cupping glasses ("rocks" glass with thick walls). The grinding machine was constantly cleaned by grinding a bit of the same sample in advance. A paper lid was immediately placed on each cup containing

ground coffee powder. Three cups containing 8.25 ± 0.25 grams coffee powder from each sample was arranged in a triangular manner (1, 2 per row) on the cupping table for evaluation. Roasted and green coffee of each sample were put beside a triangle tip and covered until after the cupping session was over to provide additional comments about the cup based on bean appearance.

6.2.2 Data collection and analysis

Data collection was done in collaboration with three experienced professional Robusta coffee organoleptic cup testers from Uganda Coffee Development Authority (UCDA). Robusta coffee cupping protocol during this exercise was developed by The Coffee Quality Institute of America (CQIA) alongside UCDA, Specialty Coffee Association of America (SCAA) and other international experts (UCTF 2008; 2009). Biochemical flavours that influence Robusta cup quality were subjectively detected, quantified and described using the cupping vocabulary.

Ground coffee fragrance was sniffed through the nose. A numeric scale of 1-10 (1=least perceived and 10=strongly perceived) was used to rate all organoleptic cup attributes; fragrance or aroma, flavour, aftertaste, salt/acid, bitter sweet, mouth feel, aftertaste and balance based on the cupper's previous experience. Defective unpleasant smell sensations were also noted and recorded. Within the 15 minutes after grinding, a ground sample infused (brewed) to minimize staling and oxidation. A ratio of 8.25 ± 0.25 grams of roasted ground powder per 150 ml of boiled water was used. Boiled mineral water (94°C =195-205°F) was poured in the cups with coffee powder. The boiled mineral water used to brew coffee was from Rwenzori Beverage Company Limited (Uganda), International Standards Organization (ISO) 9001-2000 certified Company. The water mineral composition in parts per million (ppm) comprised Sodium (9.2), Potassium (2.5), Fluoride (0.6), Chloride (4.0), Copper (0.005), Magnesium (3.7), Iron (0.04) and Calcium (10.5) with a ph of 6.9.

Soon after boiled water was poured in the ground coffee powder, coffee aroma which also helps pre-determine flavour (Clarke, 1986) was perceived by gently sniffing nasally via the nose or by having coffee in the mouth or swallowed and allowing volatile compounds drift upwards into the nasal passage. After 1-2 minutes, the crust of the brewed coffee was broken thrice using one of the preheated spoons and the major aroma details was taken and noted by positioning the nose directly over the cup. The cup was then stirred a little to ensure that all the ground coffee sunk in the bottom of the cup. Due to the high density of the medium dark roasted coffee, most of the ground coffee sunk. The spoon was rinsed in hot water after stirring or scooping from each cup and the hot water renewed as deemed necessary. After evaluating the aroma of all the cups, floating coffee was scooped out. After 5 minutes of coffee brewing and sufficient cooling, brew saltiness, acidity, flavour and aftertaste was assessed by slumming the liqour strongly in the mouth and aspirating over the entire tongue distributing tiny droplets of coffee into the throat and nasal passage. Acidity was perceived as a sharp and pleasing sweet to fruity taste akin to the bright and dry taste experienced on the back sides of the tongue while drinking red wine. Flavour was rated on how one appreciated the coffee brew taste and aroma. Aftertaste was judged as the lingering remnant sensation experienced at the back of the throat after swallowing and often changed over time. Cup evaluators rinsed their mouths between each coffee sample test.

Mouth-feel or liquor body was determined by micro fine fiber, protein and fat content in the brew. The viscosity (weight) caused by proteins and fibers and slipperiness (texture) of coffee liquor on the tongue was compared with that of pure water. The cup brew was perceived in the tongue as either sweet or bitter and more marks were scored for sweeter than bitter taste. A brew with equal intensities of flavour, aftertaste, mouth feel and bitter/sweet was quantified as a balanced cup. The sequence of rating cup attributes were based on flavour changes perceived with decreasing

temperature of the coffee brew. Each cup attribute was evaluated 2-3 while the liquor brew cooled. Additional information of evaluated samples were obtained from the green and roasted coffee samples based on appearance. Finally all flavor attributes of each sample were rated as a single overall personal judgment guided by past experience.

Total scores were obtained by summing scores for the different primary attributes. The maximum score ranged from 1-100. Data was analyzed as described in chapter 4 section 2.5. The PCA was done as described in Chapter 3 section 2.6. Like in biochemical compound analysis, a two way non parametric multivariate analysis of variance was conducted using Bray Curtis distance measure with 9999 permutations to determine significant differences among multivariate organoleptic groups.

6.3 Results

There were significant differences among evaluators for the different organoleptic cup attributes as shown in Table 6.1. Evaluator 1 and 2 rated evaluated samples significantly fragrant and aromatic. Evaluator 1 detected low flavour, mouth feel, balance and overall cup taste. As for evaluator 2, the accessions had good aftertaste and cup balance. Evaluator 2 and 3 detected saltiness, bitterness and overall low rating for the study samples.

Table 6.1: ANOVA of Robusta coffee organoleptic cup attributes by three evaluators

ANOVA statistic summary						
Cup attributes	R^2	v.r	p v	Evaluators	coefficient	p v
Fragrance/aroma	0.03	9.89	****	EV1, EV2	0.13, 0.14	**, **
Flavour	0.03	10.66	****	EV1	-0.24	****
Aftertaste	0.05	15.85	****	EV2	0.23	****
Salt/acid	0.20	76.90	****	EV2, EV3	-0.55, -0.54	****, ****
Bitter sweet	0.19	70.39	****	EV2, EV3	-0.62, -0.59	****, ****
Mouth feel	0.06	18.38	****	EV1	-0.30	****
Balance	0.14	50.73	****	EV1, EV2	-0.26, 0.22	****, ****
Overall	0.05	14.57	****	EV1	-0.25	****
Total score	0.09	31.73	****	EV2, EV3	-0.64, -0.67	**, ****
F Stat; F2, 621						

, ** significant at 0.06, 0.05, 0.0001 levels of probability

Key to Table 6.1: R^2=coefficient of multiple determination; v.r=variance ratio; p.v=probability value; coefficient=model coefficient; EV=evaluator; EV1=Fidel; EV2=Beatrice; EV3=Rita; F Stat; F2, 621= Fisher statistics, 2 degrees of freedom for factor (evaluators) =F2 and 621 degrees of freedom for cup attribute variables

The least rated organoleptic cup attribute was salt/acid with a minimum score of 6.0, maximum value of 7.67 and mean rating of 6.80 (Table 6.2). The highest minimum score of 6.5 and mean rating of 7.43 was for cup balance. Flavour mean rating was as high as 7.42. The highest maximum score of 8.67 was attained in aftertaste. With the exception of salt acid, all minimum organoleptic cup attribute values were of average rating, with a range of 61-70% (Tables 6.4 and 6.5). Except for salt acid and bitter sweet attribute, maximum values for other cup attributes were from fine coffee grade (Tables 6.4 and 6.5). Aftertaste (0.15) and fragrance/aroma (0.14) had the most organoleptic cup variance while cup balance had the least (0.08).

Table 6.2: Variability of organoleptic cup attributes derived from 201 genotypes

	Faroma	Flavour	Aftaste	Saltacid	Bsweet	Mthfeel	Bal	Overall
Minimum	6.33	6.33	6.33	6.0	6.33	6.25	6.5	6.17
Maximum	8.33	8.33	8.67	7.67	7.83	8.17	8.17	8.33
Mean	7.32	7.42	7.36	6.80	7.09	7.30	7.43	7.41
V. range	2.0	2.0	2.34	1.67	1.50	1.92	1.67	2.16
s.e	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02

Key to Table 6.4: s.e _standard error; Faroma_fragrance and aroma; Aftaste-aftertaste; Salt/acid_saltiness and acidity; Bsweet_bitter sweet; Mthfeel_mouth feel; Bal_balance

All samples were rated above fair grade (Table 6.3). There were no fine grade genotypes for acidity and liquor sweetness. Out of 208 genotypes, only 39 had very good acidity. Almost half of the genotypes had an average score rating of bitter/sweet while the other half were of very good grade. Three quarters of the genotypes were rated very good for fragrance/aroma, flavour, aftertaste, mouth feel, balance and overall cup attributes. When genotypes were rated for cup salt/acid and bitter sweet attributes, none of them attained fine grade.

Table 6.3: Organoleptic cup attribute score rating for 209 coffee genotypes

Organoleptic cup attributes	Accession score rating			
	Fair	Average	Very good	Fine
	50-60%	61-70%	71-80%	81-90%
Fragrance/aroma	0	50 (32.7%)	153 (64.9%)	5 (2.4%)
Flavour	0	29 (14%)	173 (83.1%)	6 (2.9%)
Aftertaste	0	43 (20.7%)	155 (74.5%)	10 (4.8%)
Salt/acid	0	169 (81.3%)	39 (18.7%)	0
Bitter sweet	0	107 (51.4%)	101 (48.6%)	0
Mouth feel	0	48 (23.1%)	156 (75%)	4 (1.9%)
Balance	0	19 (9.1%)	186 (89.5%)	3 (1.4%)
Overall assessment	0	27 (13%)	175 (84.1%)	6 (2.9%)

All organoleptic cup attributes had a significant and positive correlation with overall cup assessment as indicated in Table 6.4. Except for lack of significant correlation between fragrance aroma with salt acid and bitter sweet taste, all cup attributes were significantly positively correlated to each other.

Table 6.4: Relationships among coffee organoleptic cup attributes of 209 genotypes

	Faroma	Flavour	Aftaste	Saltacid	Bsweet	Mthfeel	Balance	Overall
Faroma	-							
Flavour	0.32**	-						
Aftaste	0.27**	0.75***	-					
Saltacid	0.10	0.34***	0.25**	-				
Bsweet	0.13	0.43***	0.33***	0.31***	-			
Mthfeel	0.21*	0.60***	0.54***	0.31***	0.37***	-		
Balance	0.24**	0.61***	0.57***	0.51***	0.42***	0.64***	-	
Overall	0.25**	0.72***	0.62***	0.41***	0.54***	0.73***	0.75***	-

*, **, *** significant at 0.05, 0.003, 0.0001 levels of probability

In Figure 6.1, cup balance was regarded the most important in overall cup assessment (0.90). Flavour and mouth feel were rated second in overall cup assessment. Fragrance and aroma was least considered in overall cup test (0.22).

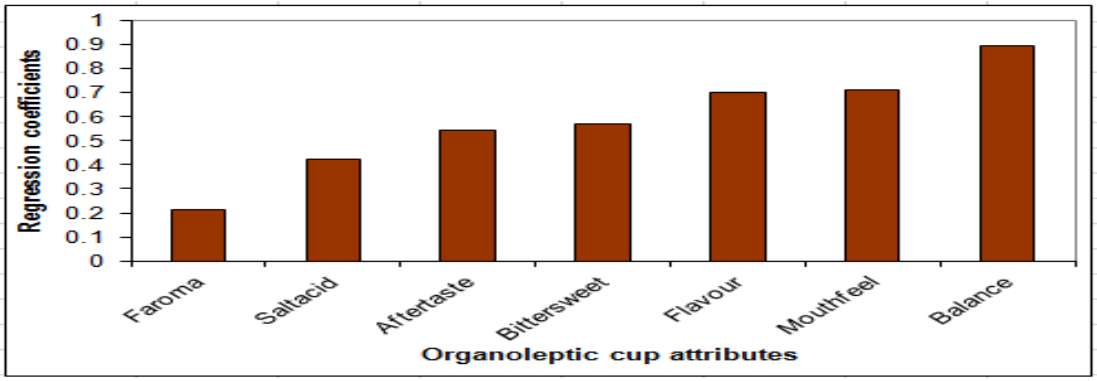


Figure 6.1 Contribution of organoleptic cup attributes to overall organoleptic cup assessment using regression coefficients

The best organoleptic cup was obtained at 1189 m above sea level from 32 year old trees (Table 6.5). Optimum cup quality was attainable at altitude range 1041-1337 m above sea level and tree age 10-54 years.

Table 6.5: Gaussian gradient and tree age for organoleptic cup attributes

Estimates	Faroma	Flavour	Aftaste	Salt/acid	Bsweet	Mthfeel	Bal
Optimum alt	1189.38 ±148.31	1188.96 ±148.06	1188.63 ±148.12	1188.62 ±148.18	1188.51 ±148.83	1188.51 ±148.66	1189.07 ±148.09
Maxm rating	8.33	8.33	8.67	7.67	7.83	8.17	8.17
Optimum age	31.67 ±21.56	31.68 ±21.54	31.68 ±21.55	31.67 ±21.48	31.73 ±21.53	31.65 ±21.52	31.67 ±21.52
Maxm rating	8.33	8.33	8.67	7.67	7.83	8.17	8.17

Hybrid types had significantly higher salt and bitterness as shown in Table 6.6. Cup acidity was detected in beans from 31-40 year old trees, height 1001-1100 m above sea level and 1201-1400 m above sea level. Altitude 1401-1500 m above sea level and Mbarara samples had a weak Robusta coffee cup aftertaste. Cup balance was significantly better at height 1300-1400 m above sea level. Collections from Masaka had a weak organoleptic cup fragrance and aroma. Hybrids from Kituza had significantly limited acidity and bitter. A sweet cup was observed from Bundibudyo, Kiboga, Kamuli and Mayuge collections. Robusta coffee cup from Bushenyi was devoid of cup balance.

Table 6.6: ANOVA of organoleptic cup attributes with Robusta coffee types, tree age, altitude ranges and locations

ANOVA statistic summary								
Cup attribute	R^2	d.f	v.r	p v	predictors	coeff	s.e	p v
Salt/acid	0.05	3,201	3.72	**	hybrids	-0.36	0.13	***
Bittersweet	0.03	3,201	1.82	ns	hybrids	-0.23	0.12	*
Salt acid	0.02	7,196	0.70	ns	31-40	0.23	0.12	**
Aftertaste	0.04	6,197	0.70	ns	1401-1500	-0.37	0.19	**
Salt acid	0.06	6,197	2.07	*	1001-1100	0.31	0.15	**
					1201-1300	0.38	0.14	***
					1301-1400	0.32	0.16	**
Balance	0.03	6,197	0.87	ns	1301-1400	0.28	0.14	**
Faroma	0.10	22,182	0.94	ns	MA	-0.34	0.15	**
Aftertaste	0.08	22,182	0.69	ns	MR	-0.34	0.18	*
Salt acid	0.10	22,182	0.96	ns	KZ	-0.42	0.15	***
Bitter sweet	0.12	22,182	1.14	ns	BU	0.28,	0.14	**
					KG	0.27	0.27	**
					KM	0.24	0.24	*
					MY	0.40	0.05	**
Balance	0.08	22,182	0.77	ns	BS	-0.22	0.13	*

*, **, *** significant at 0.07, 0.05, 0.008 levels of probability

Key to Table 6.6: ns_not significant; predictor=model coefficient; predictors=predictor variables; MA_Masaka; MR_Mbarara; KZ_Kituza; BU_Bundibudyo; KG-Kiboga; KM_ Kamuli; MY_Mayuge; BS_Bushenyi

"Erecta" and "nganda" landraces had more acid organoleptic cup as compared to the commercial and hybrid types as shown by Figure 6.2a. The "erecta" types were sweeter while the hybrids were bitter. Altitude range of 1301-1400 m above sea level produced better aftertaste while elevation of 1401-1500 m above sea level had reduced aftertaste (Figure 6.2b). Robusta coffee acidity mean values were highest at 1201-1300 m above sea level and lowest at 1501-1600 m above sea level. Organoleptic cup balance was highest at elevation 1301-1400 m above sea level and lowest at 1501-1600 m above sea level.

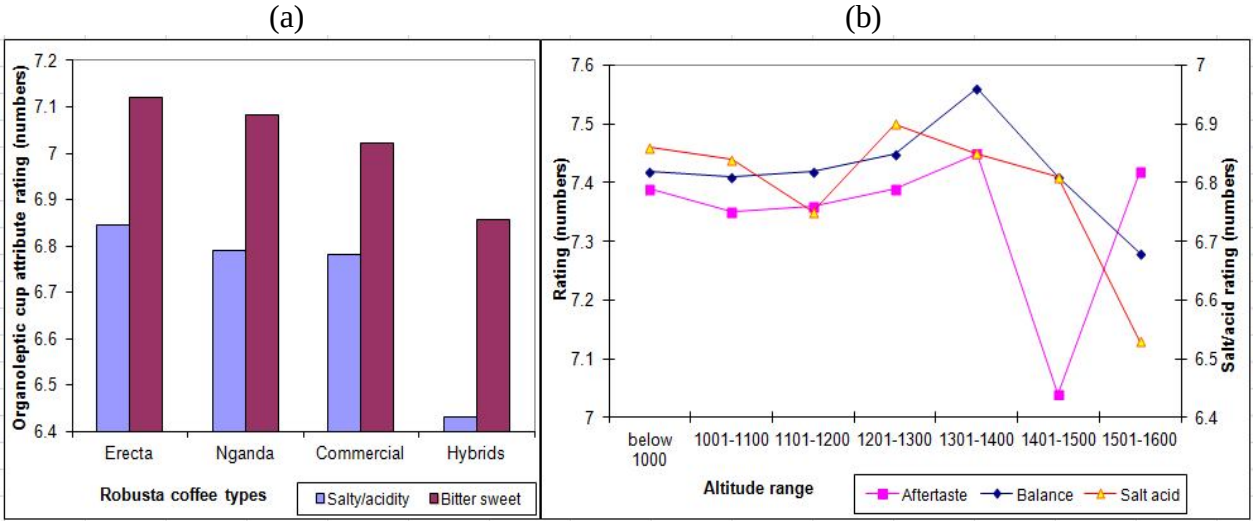


Figure 6.2: Comparison of mean organoleptic cup attributes for (a) coffee types salty/acid and bitter sweet attributes (b) altitude ranges aftertaste, balance and salty/acid

Balance and aroma were the most highly rated in all locations while salt/acidity was the least (Figure 6.3). Robusta coffee collections from Iganga, Kamuli and Rukungiri were more fragrant and aromatic while Masaka had less fragrance and aroma. As regards salt/acid character, entries from Bugiri and Kamwenge had more acidity while Kituza hybrids had the least acidity. The sweetest organoleptic cup was derived from Mayuge while the most bitter were collections from Kabarole and Kituza. Superior cup balance was obtained from Bugiri, Hoima and Kiboga while Bushenyi was regarded of least cup balance.

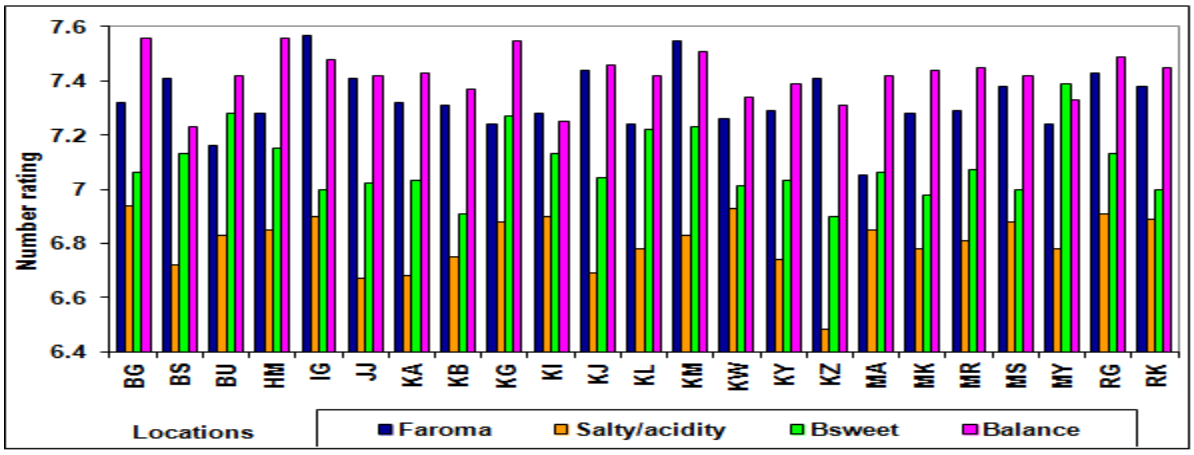


Figure 6.3: Comparison of Robusta coffee mean organoleptic cup fragrance/aroma, salty/acid, bitter sweet and cup balance at various locations

Genotypes with the best organoleptic cup qualities are shown in Table 6.7. Genotypes that excelled in flavour, aftertaste, salt/acid, mouth feel and balance were 105, originating from 24 locations with a minimum number of 2, maximum number of 7 and average of 4.38 per location. Entries that had high fragrance and aroma were 29 and were derived from 16 locations with a mean value of 1.81 per site. Characteristically sweet samples were from 26 genotypes sampled from 17 locations with an average of 1.47 samples per place. A minimum of one sample per site was recorded for fragrance/aroma and bittersweet characters with a maximum value of 4.

Table 6.7: Aggregation of genotypes based on the highest organoleptic cup attribute variance and squared cosines of observations from the principal component analysis

F	eigen value	var (%)	Cum %	variables	site	min	max	mean	sdv	total
F1	3.53	50.43	50.43	flavour, aftertaste, salt/ acid, mouthfeel, balance	24	2	7	4.38	1.66	105
F2	0.98	14.02	64.45	Faroma	16	1	4	1.81	1.05	29
F3	0.78	11.16	75.61							
F4	0.67	9.52	85.13	bittersweet	16	1	4	1.63	0.96	26

Key to Table 6.7: F_Principal component analysis factor; Var%_variance percentage; Cum%_cumulative percentage; min_minimum; max_maximum; sdv_standard deviation.

From the PCA shown in Figure 6.4, 42.92% of organoleptic cup variance was represented in factor one while factor two had 21.29%. The collections were separated into four diversity groups, two on either side of the X axis. One group comprised genotypes that were superior in fragrance/aroma, flavour and aftertaste while directly opposite were genotypes devoid of similar attributes. Another group comprised genotypes that had better cup balance, salt/acid, mouth feel and bittersweet attribute while opposite genotypes were limited in related attributes. Genotypes that had superior organoleptic cup were from different locations. Most organoleptic cup diversity was from Hoima, Iganga, Jinja, Bushenyi, Bugiri and Bundibudyo.

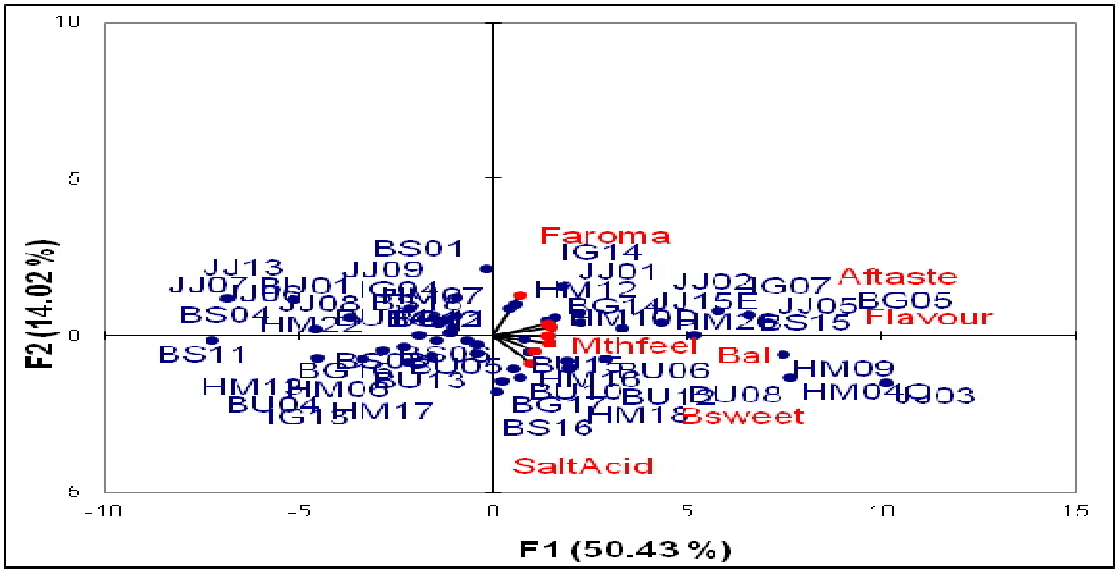
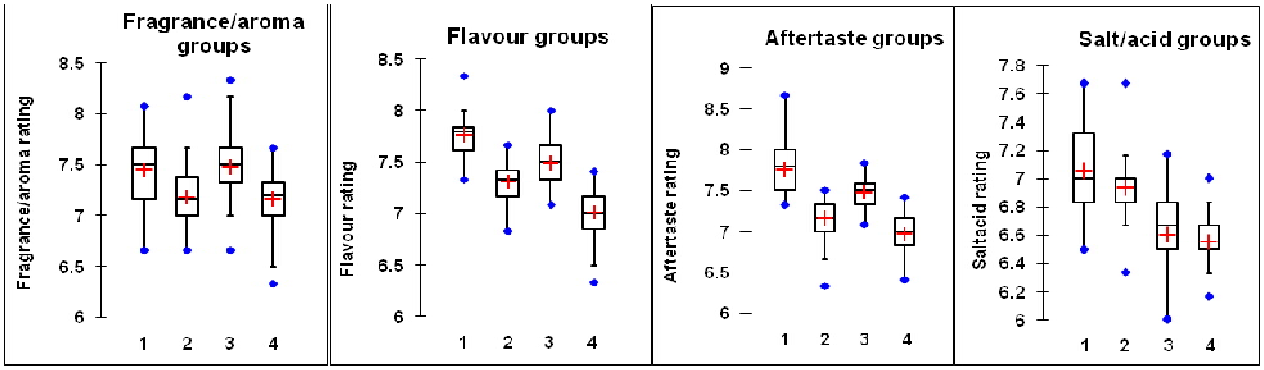


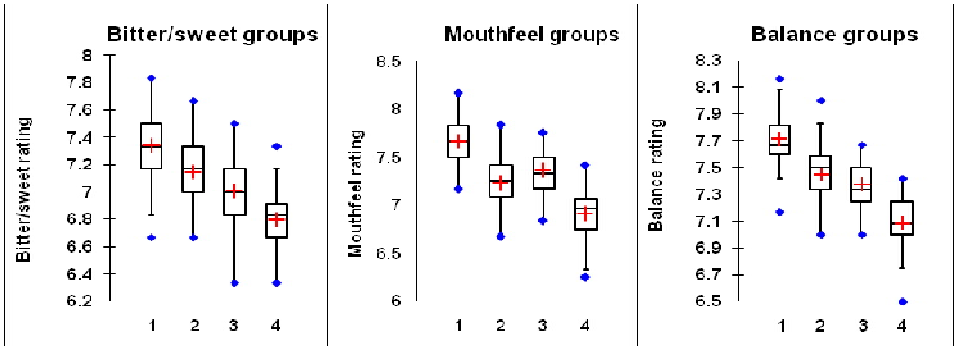
Figure 6.4: Principal component analysis of Robusta coffee organoleptic cup diversity using seven characters

Except for fragrance/aroma where group 3 had the highest mean, the rest of the attribute means were high in group 1 and lowest in group 4 (Figure 6.5 a-g). Attribute group means were placed either above or below the median. All attribute sub groups variance and inter quartile range values varied pointing out different levels of variation among diversity groups as shown in Figure 6a-g.

(a) (b) (c) (d)



(e) (f) (g)



Figures 6.5 (a-g): Description of organoleptic cup attribute diversity groups

All Mahalanobis distances were greater than 3, implying the groups were different populations (Table 6.8). Similarly like in Fisher distances, Mahalanobis longest distance was between group 1 and 4 while the shortest was amid group 2 and 3. The distance between group 1 and 2 was greater than that between 1 and 3. All Fisher distances were significantly different, implying the formed groups had different organoleptic cup attributes.

Table 6.8: Estimated Mahalanobis and Fisher distances for Robusta coffee organoleptic cup attribute groups

	Mahalanobis distances				Fisher distances				P-values for Fisher distances			
Gp	1	2	3	4	1	2	3	4	1	2	3	4
1	0				0				1			
2	11.81	0			46.18	0			<0.0001	1		
3	9.80	5.76	0		36.35	22.31	0		<0.0001	<0.0001	1	
4	35.73	9.10	11.14	0	117.04	30.97	36.20	0	<0.0001	<0.0001	<0.0001	1

Group 1 comprised genotypes that were superior in all organoleptic cup attributes (Figure 6.6 a, b). Group 2 had more acidity, sweet and balanced cup while group 3 genotypes had better mouth feel, flavor, aftertaste and fragrance (Figure 6.6 a, b). Genotypes that were inferior in all organoleptic cup attributes were placed in group 4 (Figure 6.6 a, b). The longest distance was from group 1 to 4 while group 2 and 3 were between 1 and 4.

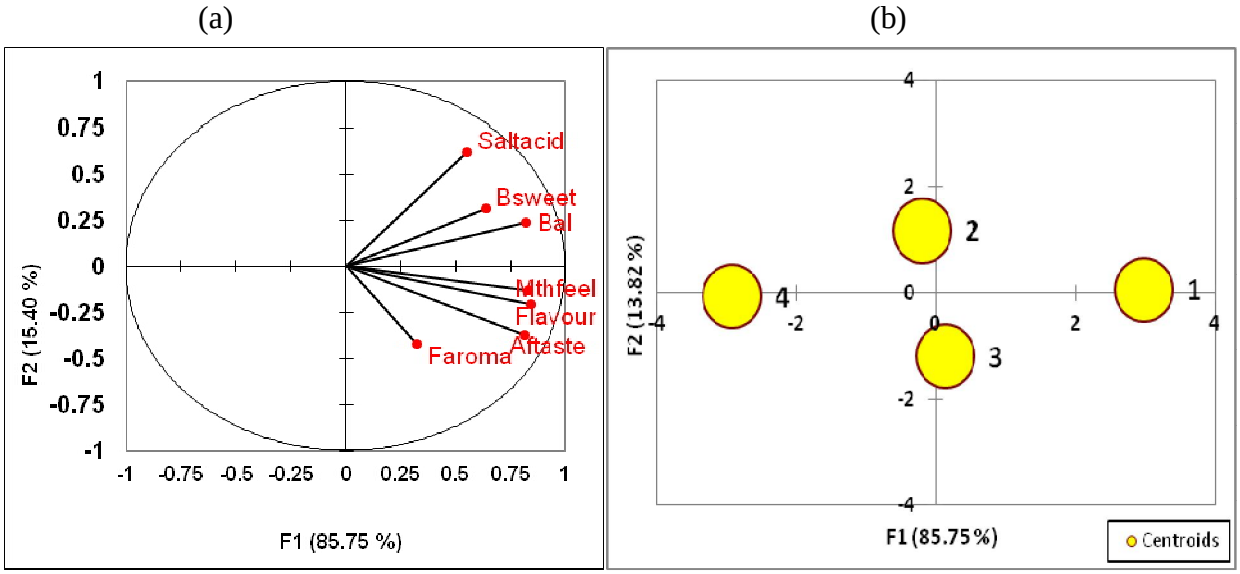


Figure 6.6: (a) Organoleptic cup attribute relationships as shown by the Principal Component Analyses (b) Organoleptic cup attribute groups from a factorial step discriminant analysis

Correct genotype group placement ranged from 88.10-96.23% with an average of 92.79% (Table 6.9).

Table 6.9 Estimated correct genotype group placement and pair wise distance comparison for farm Robusta organoleptic cup attributes

Confusion matrix						
from \ to	1	2	3	4	Total	% correct
1	50	2	2	0	54	92.59 %
2	1	54	4	0	59	93.22 %
3	0	1	52	0	53	96.23 %
4	0	1	0	41	42	88.10 %
Total	51	58	58	41	208	92.79 %

Aftertastes contributed the highest variance of 16.49% to aggregation of groups while cup balance had the least variance of 12.37% (Table 6.10). Except for fragrance /aroma factor that was highest in group 3, group 1 was superior in all organoleptic cup values. Group 4 had the least rating for all cup attributes. Group 2 and 3 cup values were intermediate.

Table 6.10: Pooled Robusta coffee organoleptic cup attribute variance contribution and mean group content derived from Bray-Curtis distance measure

	Variance		Mean group abundance			
Variable	Contribution	Cumulative %	1	2	3	4
Aftaste	0.47	16.49	7.76	7.16	7.47	6.97
Mthfeel	0.43	31.43	7.66	7.22	7.36	6.91
Flavour	0.43	46.33	7.77	7.31	7.49	7.02
Faroma	0.42	60.95	7.45	7.18	7.48	7.16
Salt/acid	0.39	74.53	7.05	6.93	6.6	6.55
Bsweet	0.37	87.63	7.34	7.14	7.01	6.79
Balance	0.35	100	7.72	7.45	7.37	7.09

One way multivariate analysis of similarity (ANOSIN) revealed a mean rank of within groups (5654), mean rank between groups (1.1E04), regression coefficient (R^2) of 0.55 and probability value of (P= 0.0001) that was similar to what Bray-Curtis distances for organoleptic cup attribute

groups obtained (Figure 6.7). In Figure 6.7, the highest mean, variance and range was realized between groups while similar measures were comparably lower in group 2 and 3. Group 1 with the best organoleptic cup test values and group 4 with the least had both similar measures of the mean, variance and inter quartile range.

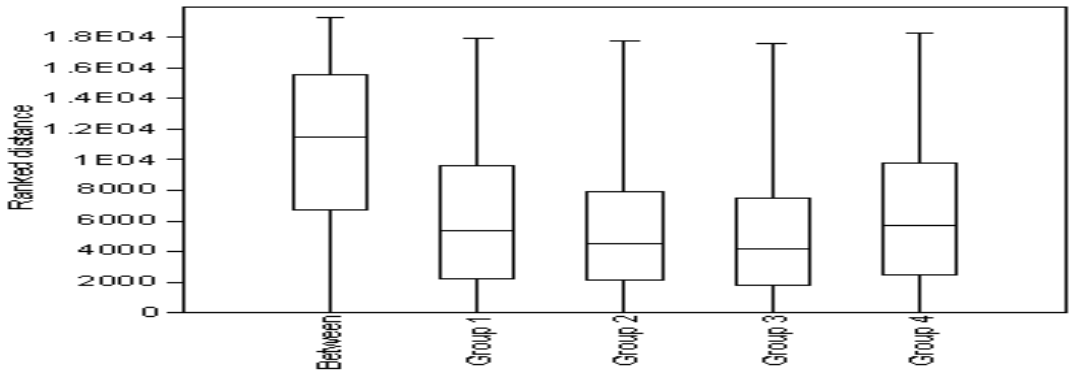


Figure 6.7: ANOSIM pair wise comparison of organoleptic cup attribute diversity using box plots

A two way non parametric multivariate analysis of variance revealed the four groups were significantly different for fragrance/aroma and flavour as shown by Table 6.11.

Table 6.11: Two way non parametric multivariate analysis of variance for the four organoleptic cup groups using Bray Curtis distance measure with 9999 permutations

Source	Sum of squares	df	Mean square	F	p
Faroma	0.0038022	2	0.0019011	1.1298	0.0003
Flavour	0.015388	2	0.007694	4.5726	0.0001
Interaction	-0.27075	4	-0.067686	-40.226	0.8791
Residual	0.33485	199	0.0016826		
Total	0.083291	207			

6.4 Discussion

The protocol used to evaluate farm Robusta coffee organoleptic cup attributes was able to detect, differentiate and segment organoleptic cup differences using their technical experience (Tables 6.1; 6.2; 6.3) The significant differences found in evaluator organoleptic cup scores reflect the subjective individual preference enriched by past experience. Individual cup taste perception and preference for varietal specific characters such as acidity, body, aroma, flavour and taste that constituted the nature and scoring of the brew implied that markets too vary and are specific. Coffee drink preference is personal and that consumers have a specific taste according to their nationality, which makes it further difficult to define organoleptic cup quality (Leroy et al., 2006). Furthermore, cup flavour in roasted coffee is reported to constitute of over 800 multiple aromatic compounds and individuals perceive them differently (Wintgens, 2004). Other than market desired roast type, blend, grind, brew, price and species, differences such as date for harvest, processing and storage which differed in evaluated accessions may have contributed to liquor differences.

Organoleptic cup characteristics were variable (Table 6.2) and assessed attributes were positively correlated either significantly or insignificantly as shown by Table 6.4, hence may provide the opportunity for improving desirable characters simultaneously. Even with the three genetic diversity groups obtained from cultivated *C. canephora* (Figures 3.4; 3.5; 3.6), sensory method still can characterize genetic diversity to a great extent. The four categories of organoleptic cup attributes obtained from the principal component (Figure 6.4) and multivariate analyses (Figure 6.6) reflected the combined effect of inherent traits and influence of the environment that is normally diverse. One of the four organoleptic cup diversity categories regarded as having the best brew had the highest ratings for fragrance/aroma, flavour, aftertaste, acidity, sweetness, mouthfeel and balance, pointing out most desirable brew group. The group with the lowest value scores were

considered to be inferior. Two groups with moderate characters differed in that one group had characteristically high acidity, sweetness and balance while the other had high fragrance/aroma, flavour, aftertaste and mouth feel. Since all organoleptic cup attribute variance contributed to group formation with close range values of 16.49% (aftertaste) to 12.37% (balance) (Table 6.10) implied that the attributes were all important in determining the quality of brewed Robusta coffee.

The fact that different groups comprised genotypes from different locations that were far apart with varying ecologies and crop husbandry point out that organoleptic variability is not isolated or restricted to any site or location (Figure 6.4; 6.7), but genetically and environmentally influenced (Leroy et al., 2006). An optimum altitude range of 1041-1337 m above sea level and tree age range of 10-54 years (Table 6.6) were estimated for the best bean organoleptic cup. The diverse organoleptic cup characteristics (Table 6.2) coupled with a wide geographical distribution (Table 6.7; Figure 6.3; 6.4) provided immense variability among Ugandan grown landraces of coffee that can be selected for quality traits and other desirable agronomic traits improvement. Organoleptic cup diversity also conforms to great range of phenotypic variability earlier reported in Ugandan Robusta coffee with a natural wide geographical distribution (Maitland, 1926; Thomas, 1940; Berthaud, 1986; Leakey, 1970).

The extensive range of commercial and fine flavours detected in green and roasted bean organoleptic cup trait variations (Table 6.2; 6.3; 6.5) gives the country an opportunity to offer a broader spectrum of flavours to markets that are characteristically diverse. Two way non-parametric multivariate analysis of variance for the four organoleptic cup groups using Bray Curtis distance revealed significant differences for fragrance/aroma and flavour (Table 6.11), which according to Tessema et al., 2011 are highly heritable attributes, therefore have a high chance of being transferred to the offspring. The least average rating of usual good quality was recorded for

all attributes with fine grade being the highest, pointing out that Uganda trades in high grade Robusta coffee (Table 6.3) more so when good harvest and processing practices are adhered to. Overall acidity had the least cup grading, an attribute that contributes to the rating of Robusta coffee (Prakash et al., 2005), despite some cultivars being rated premium grade. Of the 208 genotypes, 81% had average liquor acidity of usual good quality while 19% were of very good premium grade. Observed organoleptic variability is an outcome of out-crossing nature of *C. canephora*, in the Ugandan situation which is dominated by landraces or of wild origin that were geographically widespread.

The work reported here also showed that it was possible to select genotypes with high acidity (Figure 6.4) and that tree age, altitude and locations influenced the trait expression (Table 6.6). Significantly high cup acidity was observed from trees of 31-40 years and elevation 1201-1400 m above sea level. Trees of 31-40 years are expected to be well adapted and rooted to adequately nourish berries. Elevation of 1200-1400 m above sea level offered optimum temperatures for normal tree functioning to nourish fruits. Organoleptic cup variations such as more acidity in Bugiri, Kamwenge and sweet cup obtained from Bundibudyo, Kiboga, Kamuli and Mayuge (Table 6.6; Figure 6.3) suggest environmental influence which was not necessarily by altitude alone, but from a combination of tree and soil management effects that interacted with genotypic traits. Research hybrid selections from Kituza had dense and large beans (Table 4.1.5) with low acidity (Table 6.6). Similarly, Blue Mountain Arabica coffee is known to produce outstanding coffee quality from Jamaica but not in East Africa.

Acidity is a characteristic of coffees grown at high altitudes as noted in the Ugandan case where Robusta coffee is commonly grown at height range 1000-1500 m above sea level and in mineral rich volcanic soils (Wrigley, 1988). A part from much acidity, coffee from high elevation tends to

have high aroma and flavor, small dense and harder beans with a narrow roasted bean centre cut. At very high altitudes, such as 1500-1600 m above sea level in the Ugandan case, beans had low acidity (Table 6.6). Coffee acidity is highly valued especially in Central America (Leroy et al., 2006). Incomplete bean development could occur in higher altitudes such as 1401-1500 m.a.s.l (Table 6.6) where aftertaste rating was low as a result of inadequate bean nutrition caused by leaf distortion and discoloration, a condition referred to as "hot and cold effect". Like in other crops, hot and humid conditions yield lower flavour and bean structure.

Perceived acidity in coffee does not necessarily correlate to the pH of coffee, but results from acids present in the brew, best attained from fine ground roasted coffee with 94°C water temperature for 5 minutes (ICO, 1991). Coffee acidity, aroma and sweetness decrease as the bean roast darkens. Griffin et al., 1999 observed significantly increased extractable phosphate at the start of roasting. Other than roast time and extraction, mineral content of water, water temperature, evaluation time, grind size and brew method influence acidity levels. Increased body of dry processed coffees is believed to mask the acidity in the cup.

The work here revealed that farmer coffee cup acidity had positive correlation coefficients with a sweet cup, good mouth feel, cup balance and overall cup (Table 6.4). Other researchers such as Moschetto et al., (1996) also reported linear correlation coefficients between preference and acidity and aroma in Robusta coffee hybrids and commercial clones. But, Decazy et al., 2003 associated sensory quality with high altitude, well distributed annual rainfall of less than 1500 mm and optimum roast time per variety. Because of the easiness to define and select, acidity and aroma can be used to identify genotypes with desirable Robusta organoleptic quality (Leroy et al., 2006).

Whereas Robusta is generally known to be less aromatic and richer in caffeine than Arabica coffee (Charrier et al., 1988), about 65% of assessed genotypes had very good rating for fragrance/aroma (Table 6.3) as well as collections from Iganga, Kamuli and Rukungiri (Figure 6.3). Coffees rich in sucrose and trigonelline have been associated with better aroma (Davrieux et al., 2003; Charrier et al., 1985; Vaast et al., 2006). In Chapter 5, where biochemical compounds were evaluated (Table 5.1; 5.2) sucrose range was wide (2.48-7.34) and the maximum concentration values were higher than those reported by Ky et al., 2001a (0.75-1.24) and Wintgens, 2004 (0.60-1.70) (Table 5.2). The minimum trigonelline content (0.66-1.03) was comparable to those of Ky et al., 2001a (0.75-1.24) and Wintgens, 2004 (0.60-1.70) with the lowest maximum. According to Tessema et al., 2011, aromatic intensity, flavour, bitterness, shape and make, average bean weight, dry matter, ash, protein and fat were highly inherent, hence can be transferred to the progeny. Whereas Ky et al., 2001b indicated high maternal cytoplasmic inheritance of 71% for trigonelline, Montagnon et al., 1998 found intermediate narrow sense heritability of 38% for trigonelline and 11% for sucrose.

In Table 5.6, loam soils produced beans with more sucrose while those from clay soils were deficient in trigonelline and fat content, probably producing beans with reduced fragrance and aroma. The low organoleptic cup fragrance/aroma and the significantly low roast bean volume increase realized in the Masaka collections resulted most likely from incompletely developed beans that did not attain maximum fragrance/aroma (Table 6.7). Except for the association between fragrance/aroma with salt/acid and bitter sweet that were not significant, flavor, aftertaste, mouthfeel, cup balance and overall assessment gave positive significant correlation coefficients (Table 6.4). It therefore implied that specific biochemical compounds that generated flavor and aroma did not necessarily generate cup saltiness or acidity while biochemical compounds that

influenced flavor, aftertaste, mouthfeel, cup balance and overall assessment had a role in each of the attributes.

Over 83% of farm Robusta coffee collections had a flavour rating of very good equated as premium (Table 6.3). All cup attributes were positively associated with flavor (Table 6.4). Compounds that determine coffee quality arise from precursors in green beans (Thomas, 1935; Davrieux et al., 2003). Fat content, a trait of high heritability (Montagnon et al., 1998) helps fix the flavour compound formed during roasting (Tessema et al., 2011; Davrieux et al., 2003; Charrier et al., 1985). The Ugandan *C. canephora* had high fat content (Tables 5.1; 5.2). The four basic flavours used in sensory evaluation to determine defects more easily and capture promotional opportunities include acidity, sweetness, saltiness and bitterness are used. Contrary to the general view that Robusta coffees are weak flavoured with low acidity, have neutral to harsh/bitter taste with cereal notes and a thick body, fine Robusta coffee were detected in this study (Table 6.3; Figure 6.4). Many espresso blends use Robusta for its flavour strength and crema (Clifford et al., 1985). Other than extraction, coffee flavor is affected by factors like roast, roasted bean grind size, mineral content of brewing water, water temperature, evaluation time, brewing procedure as well as the decaffeination process.

About 75% of study samples had a premium rating for aftertaste (Table 6.3) although at elevation 1401-1500 m above sea level and Mbarara, brew aftertaste was weak (Table 6.6). Total dissolved solids give a bitter cup while a medium roast increased acid content and a potent aroma compared to dark roast as also reported in Maier, 1987. Caffeine and chlorogenic acids though higher in Robusta coffee with lower trigonelline content compared to Arabica coffee (Table 5.2), contribute to the bitter and astringent taste (Ky et al., 2001a). According to Barbosa et al., 2012, trigonelline, caffeine and chlorogenic acid particularly acid-5-cafeiolquinic helps discriminate cup qualities.

High potassium in Robusta coffee brew produces brackish (high saltiness and un pleasant aroma) aftertaste whereas low amounts are savory (low saltiness and pleasant aroma). Lower quantities of potassium and caffeine made the coffee brew taste coarse and harsh (Clarke, 1986).

None of the factors, namely, coffee types, elevation, tree age, soil texture or location generated significant differences in mouthfeel (Table 6.6). Since mouth-feel or liquor body was determined by micro fine fiber and fat content in the brew, it therefore implied all genotypes had similar quantities of micro fine fiber and fat regardless to the factor evaluated. From Table 5.2, Ugandan samples fat content ranged from (10.44-15.94) and were higher than those reported by Wintgens, 2004 for both Robusta (7.0-11.0) and comparable to Arabica (13.0-17.0) coffee. Furthermore 75% of evaluated genotypes had premium rating for mouth feel (Table 6.3). Also in Figure 6.1, significant effect of fat to overall cup was reflected by similar rating of (r.c=0.70) by flavour and mouth feel that followed cup balance (r.c=0.90). High fat content has also been associated with organoleptic cup preference and high altitude (Decazy et al., 2003). Brew colloids that form when oils coagulate around fibers suspended in the brew determine coffee brew weight (micro fine fiber) particles and slipperiness texture (oil) in the tongue.

The highest mean rating of 7.43 (Table 6.2) with 89.5% of genotypes graded premium (Table 6.3) was observed for cup balance character. Elevation of 1300-1400 m above sea level and the locations of Bugiri, Hoima, and Kiboga had the best cup balance while altitude of 1501-1600 m above sea level and Bushenyi had the least mean values (Table 6.6). Regression coefficients revealed cup balance was the most important in determining overall cup (Figure 6.1) and Ugandan Robusta coffee has internationally been rated as mild (UCTF, 2008; 2009). A balanced cup in this regard would reflect a complementary synergistic combination of flavor, aftertaste, mouth feel and

bitter/sweet aspect ratio which is soft, pleasing with a delicate taste derived from acidity and sweet coffee fruit acids with high sugars levels that dominated Ugandan traded coffee.

The “nganda” and “erecta” landraces had significantly more acidity and were sweeter than the research elite commercial and hybrid types (Figure 6.2). Earlier studies that compared different varieties based on organoleptic evaluation and several scientific procedures indicated that similarities and differences were attributable to genetic traits (Bertrand et al., 2006; Dessalegn et al., 2008). The impact of reduced genetic variability during the selection process to improve other desirable agronomic traits could detrimentally result to genotypes like hybrid types that were found here with significantly less acidity and bitter taste (Table 6.6; Figure 6.2). High levels of potassium have been associated with salty liquor while low concentrations resulted to pleasant liquor. Low potassium along with caffeine also produced bitter, coarse and harsh brew (Maier, 1987). The consistently uniform and bright clean cup liquor among evaluated samples reflected Robusta coffee characteristic, presence of quinic acid and well processed beans.

CHAPTER SEVEN : DIVERSITY OF *Coffea canephora* NUTRIENT ELEMENTS AS MEASURED BY ENERGY DISPERSE X-RAY FLORESCENCE

7.1 Introduction

Coffea thrives between latitude 25^0N and 25^0S and requires specific environments for production and bean quality. In Uganda, Robusta coffee is grown in a diversity of soils that range from red sandy clay or gravelly loam that may contain murrum or soft laterite with climate being a major distribution factor (Wrigley, 1988). Ideal soils for coffee should be deep (at least 75cm), permeable for good drainage, slightly acidic in reaction (6.0-6.5 pH), porous to allow gaseous exchange and rich in organic matter content (>2.5 per cent organic carbon) (Wrigley, 1988). High soil organic content (6.66-17.8%) and acidic soils of pH 5.1-6.8 may promote Robusta coffee growing but due to low soil nitrogen, phosphorus, calcium, magnesium and exchangeable bases, crop yields may remain low (Zake et al., 1996). Liming can be used to restore acidity while soil fertility can be amended using organic or inorganic fertilizer or in combination and rain. Other soil management practices such as mulch, live bands may be co-opted.

Trace mineral content of plant tissue or foods reflect the trace element concentration in the soil in which they are grown for example high zinc ions content in soil enables the plant to have more zinc compared to the zinc-deficient soils depending on the species and plant genotype (Grusak et al., 1999). Fertile soils contribute to coffee bean size, weight and quality of final product (Bertrand et al., 2006). Different coffee species may respond differently in traits such as production capacity, bean quality and disease tolerance when grown in different environments. Field composition and productivity depends on the physical and chemical properties of soil which relates to the biological, geochemical cycles (Slagle et al., 2004), anthropogenic factors like deforestation and management. Macronutrients are required in greater amounts than micronutrients for normal plant growth.

To track and quantify nutrient elements, different approaches have been used of which X-ray is one of them. X-ray spectrometric techniques are sensitive and rapid quantitative and qualitative micro analytical fluorescent (secondary) techniques that can be used to determine the composition and physical structure of elements (FitzGerald et al., 2005). In most cases XRF is a non destructive analytical technique and process control tool in many extractive and processing industries and is suitably used in gas, solids, liquids and powder analysis. Anderson et al., 2002 determined nutrient concentrations and geographical origin of samples using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Analysis of Arabica and Robusta green beans using ICP-AES found significantly higher potassium and copper in Robusta coffee and manganese in Arabica coffee (Gure, 2006). Potassium, manganese and copper were suggested as chemical descriptors for differentiating roasted Arabica and Robusta coffee.

In this study, Energy Disperse X-Ray Fluorescence (EDXRF) was used to evaluate the element composition and concentration of soil, leaf and green beans. EDXRF spectrometer instrument weighs about two kilograms and has a simpler mechanical design that is easy to use. Total reflected light from XRF is developed and incorporated within the instrument for Ultra trace determinations of substances. Unlike x-ray tubes that generate high power monochromatic X-ray beams, EDXRF instrument is capable of generating and using tiny x-ray tubes or gamma sources (electron (beta) or proton (alpha) at high speed (Brower, 2003; Fitzgerald et al., 2008; FitzGerald et al., 2005. EDXRF simultaneous detects sample elements making it a cheaper option.

EDXRF is unable to detect X-rays from light elements across the periodic table from sodium (z=11) to Uranium (U), whereas WDXRF stretches down to beryllium (z=4). Problem of matrix effect, low resolution and count rate with a long pulse time shaping process or poor statistics makes EDXRF inferior for high precision analysis. However, by maintaining the detector at low

temperatures, reducing multi proton pace, setting low conductivity with reduced tube current gives more time for pulse shaping and better resolution. Using monochromatic primary energetic radiation from a secondary target with a solid state disperse detector improves matrix effects. Analyzing samples with well characterized shape, surface as well as scanning study material reflection angle for density, roughness, thickness and profiling depth help reduce matrix effects. XRF benefits also outweigh the relatively long time required to prepare adequate volume of a sample, destructive grinding and pressing to make a pellet or fusion with inert material to form a glass bead (FitzGerald et al., 2008).

Determining nutrient status in the soil, leaf and green bean provides indicative information on soil and plant nutrient status and identifies the magnitude of existing nutrient limitations in a cost effective manner (Lifang et al., 2000). Inherent and environment characteristics influence not only nutrient element differences in soil but also the chemistry of plant tissue. To identify Robusta coffee genotypes with desirable green been nutrient elements that produce high cup quality, the coffee breeding program in Uganda ought to rely on the knowledge of different environmental effects on green bean nutrient element composition which at the moment is lacking. Pre harvest factors that influence cup quality like nutrient element composition and interactions in soil to leaf and bean have been overlooked. The aim of the research therefore was to determine how soil nutrient elements are distributed in the leaf, green bean of *C. canephora* in the various growing locations.

7.2 Materials and Methods

7.2.1 Soil, Robusta coffee leaf and green bean sample collection

Leaf and soil samples were harvested from 67 tagged trees whose ripe cherry had been picked for bean quality evaluation in 23 locations and on stations of Kawanda and Entebbe (Table 4.1). To

sample soil, surface litter was removed without scraping off top soil. None of the Robusta coffee fields had apparent fertilizer remains as per observation and farmers communications. An auger was used to drill up to a depth of 30 cm in three triangular spots located 30cm-150 cm tree radius and was cleaned after each sampling. Soil samples were placed in poly bags and labeled. Physiologically mature leaves numbering 6-10 were harvested near or within ripe cherry clusters with hands kept free of contaminants. Leaves were packed in paper sample bags labeled and left aerated until they dried. Wet soil samples were initially sun dried before air drying and making a compost sample for each tree.

7.2.2 Sample preparation, spectra data collection and analysis

About 20 grams of frozen green bean were initially ground using a nitrogen pre-cooled motorized grinder at NaCRRI. Dried soil and leaf were ground to fine particle size using a motorized grinder in the University of Nairobi, Department of Nuclear Science and Technology. The pre ground coffee green powder was further crushed to finer particles using a hand motor and pestle. Ground samples were sieved using 0.1 mm screen size and fine powder of 0.3-0.5 grams was weighed using AT460 Delta-Range balance. A compacting machine pressed fine powder to homogenous flat circular discs (pellets) of diameter 20-50 mm with sufficient thickness to absorb the entire X-Ray florescence primary beam, including lighter elements which omit fluorescence of a few micrometers sample depth. X-ray was calibrated using a representative soil, leaf and green beans sample of accurately known element concentration to enable calculate investigated sample element concentration. Sample pellets were randomly picked and geometrically placed at a standardized small distance on a high precision motorized sample stage located in a containment box that acted like a vacuum by sustaining x-ray energy. The sample stage was positioned accurately beneath the

x-ray tube window in line with the detector assembly connected to liquid nitrogen (-197^{o}C). Liquid nitrogen limited lithium atoms from drifting or migrating.

To reduce the effect of sample irregularities, the sample was automatically span at 5-20 rotations per minute (rpm). Individual sample pellets were then scanned by bombarding with x-rays of Cd-109 (cadmium) radioisotope source. To minimise loss of fluorescent X-ray energy, the semi conductor detector which measures the entire energy spectrum was simultaneously placed closer to the sample. Canberra multi channel analyzer and spectral data processing software unit MCA (S-100) was linked to a personal computer. Sample spectra multiple peaks for different energy intensities were visualized on the computer monitor as a plot of specific element distribution/composition and concentration by an in built colour video cameras. Multiple elements in the sample were imaged concurrently and information from all detectable elements was captured simultaneously. The data generated was pre-amplified, amplified, stored and analyzed using AXIL and QAES computer software. In pre-amplification, the burst of electrons were converted into signals of elements which were further amplified and transformed from analog (not quantified) to quantified values in parts per million (ppm). The data was analyzed as in Chapter 4 section 3.2.

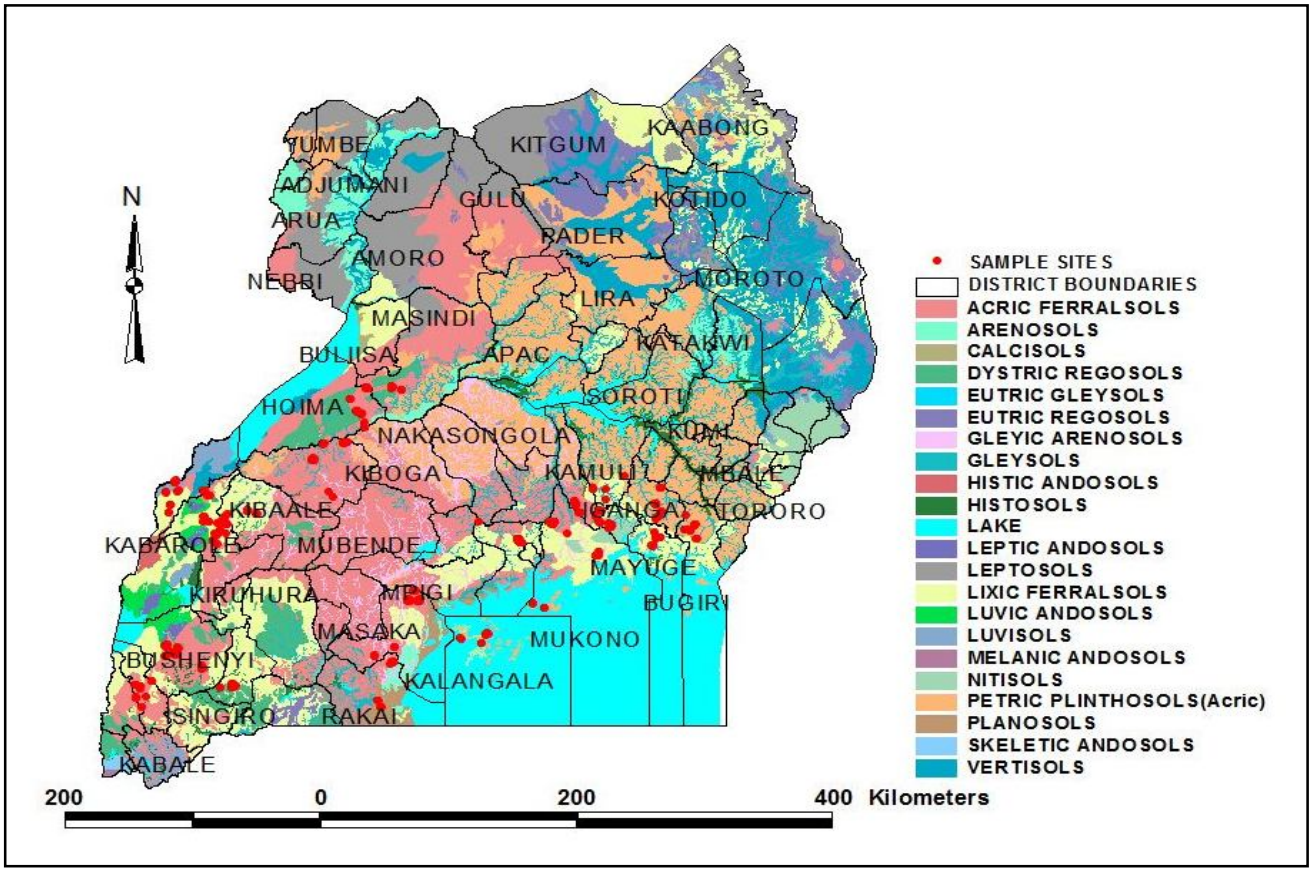


Figure 7.1: Map of Uganda soil types and study sample sites

7.3 Results

The nine nutrient elements consistently detected in the soil, leaf and green beans were potassium (K), calcium (Ca), tin (Ti), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), lead (Pb) and bromine (Br) (Table 7.1). Manganese and iron minimal values were detectable in soil. Potassium, calcium, manganese, iron, copper, zinc, lead and bromine were detected in the soil and also found in the leaf and green bean. The concentrations of manganese, iron, zinc and lead decreased progressively in the soil, to the leaf and eventually to the green beans. The mean concentration of potassium in the soil was 8206.2 ppm compared to that of 911716.4 ppm in the leaves and 12904.4

ppm in the green bean. Calcium in the soil had a mean value of 4939.3 ppm but the value increased to 13343.9 ppm in leaves and reduced to 12904.4 ppm in the green bean. Copper and bromine concentrations in the green beans were 12.8, 1.7 ppm respectively but were 10.0, 1.5 ppm respectively in the leaves.

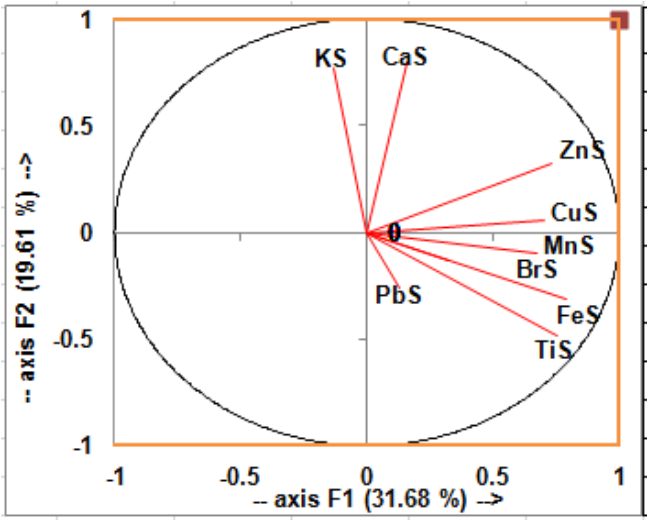
Table 7.1: Nutrient elements concentrations in ppm from soil, leaf and green bean in 67 farms

Element	Soil		Leaf		Green bean	
	Mean	Sdev	Mean	Sdev	Mean	Sdev
K	8206.2	6757.6	11726.4	4004.6	12904.4	3746.4
Ca	4939.3	4461.5	13343.9	4722.9	1273.9	770.2
Ti	8744.6	2650.4	212.3	1212.3	0.0	0.0
Mn	1823.0	1063.1	96.6	231.4	2.4	7.1
Fe	55111.1	26062.1	1444.1	6601.5	48.0	20.3
Cu	13.6	33.5	10.0	14.4	12.8	9.2
Zn	74.4	48.3	4.2	18.8	1.0	2.4
Pb	36.8	23.7	2.0	6.2	1.4	3.1
Br	12.6	12.7	1.5	5.0	1.7	4.6

Key to Table 7.1: K=potassium; Ca=Calcium; Ti= Tin; Mn=Manganese; Fe=Iron; Cu=Copper; Zn=Zinc; Pb=Lead; Br=Bromine

Relationships of soil elements using the PCA and correlation coefficients are shown in Figure 7.2. Total variance for principal component 1 and 2 was 52.29%. Potassium not sifnficanttly different from calciumwas positively associated with calcium. Lead was inversely related to soil potassium and calcium but not to significant levels. From the coefficients of determination, potassium was significantly and negatively correlated with tin and iron. Calcium was positively correlated with manganese and zinc. Tin was positively and significantly associated with manganese, iron, zinc and bromine. Manganese had a significant positive correlation with iron and zinc. Meanwhile iron was positively and significantly correlated with copper and zinc.

(a)



(b)

Association		r	R^2	P value
KS	TiS	-0.51	0.26	0.0001***
KS	FeS	-0.35	0.12	0.004**
CaS	MnS	0.24	0.06	0.05*
CaS	ZnS	0.4	0.16	0.001**
TiS	MnS	0.34	0.12	0.005**
TiS	FeS	0.59	0.35	0.0001***
TiS	ZnS	0.25	0.06	0.04*
TIS	BrS	0.4	0.16	0.001**
MnS	FeS	0.37	0.14	0.002**
MnS	ZnS	0.47	0.22	0.0001**
FeS	CuS	0.24	0.06	0.05*
FeS	ZnS	0.27	0.07	0.03*

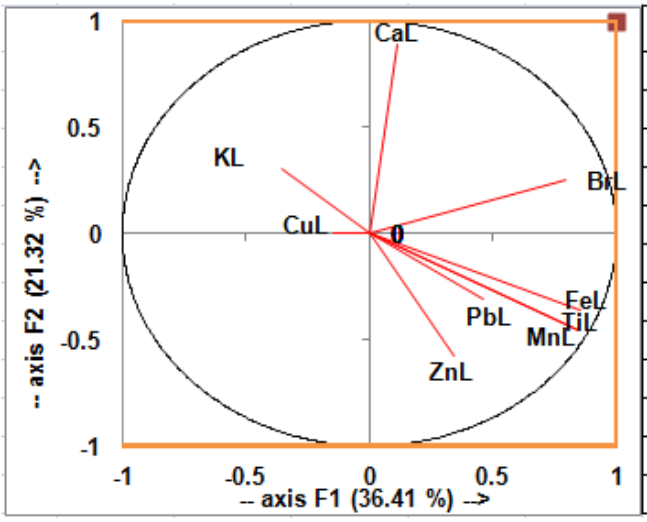
Figure 7.2: Relationships and coefficients of determination for soil elements

*, **, *** significant at 0.05, 0.004, 0.0001 levels of probability

Key to Figure 7.1: r= correlation coefficient; R^2=Coefficient of determination; p value=probability value; Letter S attached to element abbreviations imply soil element.

Relationships of leaf elements using the PCA and correlation coefficients are shown in Figure 7.3 a, b. Based on the PCA, zinc, manganese, lead, tin and iron were positively correlated and inversely related to potassium and copper (Figure 7.3a). From Figure 7.3b, potassium was significantly and negatively correlated with zinc. Calcium had a significant negative correlation with tin and zinc. Tin was positively correlated with manganese, iron, zinc, lead and bromine. Manganese was positively and significantly associated with zinc and bromine. Zinc was positively and significantly correlated with lead and bromine.

(a) (b)



Association		r	R^2	P value
KL	ZnL	-0.26	0.07	0.04*
CaL	TiL	-0.31	0.09	0.01*
CaL	ZnL	-0.26	0.07	0.04*
TiL	MnL	0.25	0.06	0.04*
TiL	FeL	0.26	0.07	0.03*
TiL	ZnL	0.59	0.35	0.0001***
TiL	PbL	0.37	0.13	0.002**
TiL	BrL	0.55	0.3	0.0001***
MnL	ZnL	0.28	0.08	0.02*
MnL	BrL	0.25	0.06	0.04*
ZnL	PbL	0.4	0.16	0.001**
ZnL	BrL	0.38	0.14	0.002**

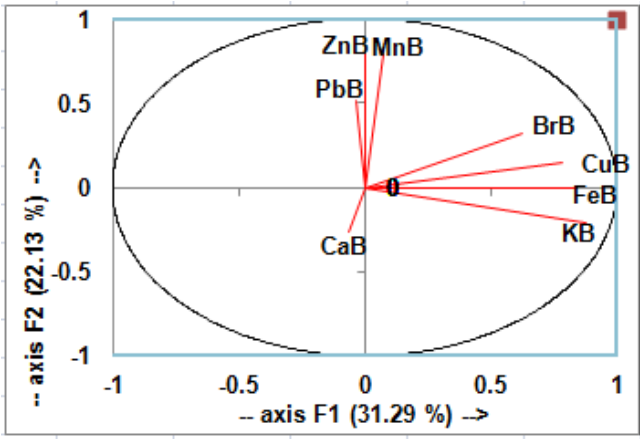
Figure 7.3: Relationships and coefficients of determination for leaf elements

*, **, *** significant at 0.05, 0.004, 0.0001 levels of probability

Key to Figure 7.3: Letter L attached to element abbreviations imply leaf element

Relationships of green bean elements using the PCA and correlation coefficients are shown in Figure 7.4. All green bean element relationships were significantly different. Calcium had an inverse insignificant correlation with lead, zinc and manganese Potassium had a significant positive correlation with calcium, iron and copper. Manganese was significantly and positively related to zinc, lead and bromine. Meanwhile a significant positive correlation existed between iron and copper. Zinc was positively and significantly correlated with lead and bromine. Lead was positively and significantly different from bromine.

(a)



(b)

Association		r	R^2	p value
KB	CaB	0.34	0.11	0.006**
KB	FeB	0.54	0.29	0.0001***
KB	CuB	0.41	0.17	0.001**
MnB	ZnB	0.46	0.21	0.001**
MnB	PbB	0.4	0.16	0.001**
MnB	BrB	0.38	0.14	0.002**
FeB	CuB	0.39	0.15	0.001**
ZnB	PbB	0.43	0.19	0.001**
ZnB	BrB	0.26	0.07	0.03*
PbB	BrB	0.47	0.22	0.0001***

Figure 7.4: Relationships and coefficients of determination between green bean elements

*, **, *** significant at 0.05, 0.004, 0.0001 levels of probability

Key to Figure 7.4: Letter B attached to element abbreviations imply green bean element.

Table 7.2 shows regression statistics of soil elements on green bean elements. Soil bromine had significant positive effects on green bromine while low manganese content significantly reduced bean iron. Lowing soil tin significantly increased bean managanese. Although fitted terms of soil nutrient elements collectively did not reflect significant effects on bean elements, significant effects of low soil tin increased bean bromine; high soil calcium increased bean bromine while high soil bromine increased bean bromine. Bean element variability explained by soil elements ranged from 5.5-10.9%.

Table 7.2 Regression statistics of soil and leaf elements on green beans from 67 farms

Regression statistic summary							
BE	SE	coefficient	s.e	v.r	p value t-test	R^2(%)	
BrB	TiS	-6E-4	2E-4	1.75	ns	**	7.4
CaB	CaS	0.05	0.02	1.55	ns	**	5.5
FeB	BrS	0.48	0.20	2.15	**	**	10.9
FeB	MnS	-6E-3	0.003	2.15	**	**	10.9
KB	BrS	72.7	38.2	1.66	ns	*	6.6
MnB	TiS	-8E-4	4E-4	2.13	**	**	10.7
Fstat; F7, 59							

*, **, ***, **** significant at 0.06, 0.05 levels of probability

Fitted terms: Constant + BrS + CaS + PbS + MnS + TiS + CuL + TiL

Key to Table 7.2: BE=bean elements; SE=soil elements; coefficient=regression coefficient/slope; s.e=standard error; d.f=degrees of freedom; v.r=variance ratio; F value=Fisher probability test; t-test=Student t test; R^2= coefficient of determination/percentage variance explained; ns=not significant; Fstat; F7, 59= Fisher statistics; 7 factors and 59 variable degrees of freedom.

Table 7.3 shows a regression of soil elements on leaf elements. The highest leaf copper variability of 17.1% was explained by significantly high soil lead, low soil potassium and soil manganese. High concentrations of soil bromine significantly reduced leaf potassium. Increasing soil tin significantly increased leaf zinc. Even with insignificant fitted terms, soil lead, tin and copper elements had significant effects on leaf elements. Leaf element variability explained by soil elements ranged from 3-17.1%.

Table 7.3 Effects of soil elements on leaf nutrient elements

Regression statistic summary							
Leaf elements	soil elements	coefficient	s.e	v.r	F p	t p	R^2(%)
BrL	PbS	-0.07	0.03	1.62	ns	**	5.3
CuL	PbS	0.17	0.08	3.26	***	**	
	KS	-7E-4	2E-3	3.26	***	**	17.1
	MnS	-4E-3	2E-3	3.26	***	**	
FeL	TiS	1.0	0.41	1.29	ns	**	2.6
KL	BrS	-132.8	40.30	2.43	**	***	11.5
MnL	TiS	0.04	0.01	1.76	ns	***	6.5
	CuS	-1.76	0.97	1.76	ns	*	6.5
TiL	TiS	0.18	0.08	1.34	ns	**	3.0
ZnL	TiS	0.002	0.001	2.15	*	**	9.5
Fstat; F6, 60							

*, **, ***, **** significant at 0.07, 0.05, 0.008 levels of probability

Fitted terms: Constant + PBS + BRS + TIS + KS + MNS + CUS

Key to Table 7.3: Fstat; F6, 60= Fisher statistics; seven factors, 59 variable degrees of freedom.

The effect of increasing coffee tree age and elevation on leaf and green beans element occurrence and quantity is shown in Table 7.4. Bean manganese significantly increased with increasing tree age. Elevated areas had significantly high amounts of zinc in green beans. Altitude accounted for 11.8% bean zinc variability while age of trees accounted for only 5.5% of bean manganese variability.

Table 7.4 Effects of altitude and tree age on soil, leaf and green bean elements

		Regression statistical summary					
Bean element	predictor	coefficient	s.e	v.r	F p	t p	R^2(%)
MnB	age	0.10	0.04	2.94	*	**	5.5
ZnB	altitude	0.006	0.002	5.42	***	***	11.8
Fstat; F2, 64							

*, **, *** significant at 0.06, 0.05, 0.009 levels of probability

Fitted terms: Constant + altitude + tree age

Key to Table 7.4: Letters L and B attached to element abbreviations imply leaf and bean elements; predictor=independent or predictor variable.

Table 7.5 shows differences in coffee types, altitude range and soil texture for soil elements. Commercial varieties had high concentrations of soil tin and lead (Table 7.5). Soil lead significantly increased at elevation 1101-1300 m a s l. Loam clay soil texture had significantly reduced soil calcium content and more soil copper.

Table 7.5: ANOVA for soil nutrient elements with coffee types, altitude range and soil texture

		ANOVA statistic summary						
SE	R^2	d.f	v.r	p v	category	coeff	s.e	pvalue
TiS	0.06	2,64	2.20	ns	commercial types	2100.3	1018	**
PbS	0.11	2,64	4.13	**	commercial types	24.49	8.85	***
PbS	0.14	5,61	1.92	ns	1101-1200 m.a.s.l	27.6	10.2	***
					1201-1300 m.a.s.l	28.8	11.5	***
CaS	0.17	4,62	2.44	**	loam clay	-1398	624	**
CuS	0.23	4,62	3.57	***	loam clay	21.76	8.9	***

*, **, ***, **** significant at 0.09, 0.05, 0.004, 0.0006 levels of probability

Key to Table 7.5: SE= soil elements; coeff=model coefficient; p value=probability value; s.e=soil elements.

Table 7.6 shows locational differences for soil elements. Based on the model coefficients, potassium was significantly low in Kabarole and Kibale while calcium was high in Bundibudyo, Iganga, Kyenjojo and Rukungiri. Tin and copper were significantly high in Jinja. Iron was significantly different in all study locations. Zinc nutrient was significantly low in Hoima, Kiboga, Kibale, Kamuli and Mayuge. Bugiri, Bushenyi, Iganga and Kayunga were deficient of lead whilst Kamwenge and Kayunga had significant amounts of bromine.

Table 7.6: ANOVA of soil elements with 23 locations

ANOVA statistic summary							
SE	R^2	v.r	p v	location	coefficient	s.e	p v
KS	0.54	2.34	***	KB, KI	-8706,-11276	4293, 4293	**, **
CaS	0.52	2.18	**	BU, IG	7846, 6853	3086, 3450	**, **
				KY, RG	9267, 8505	2886, 2886	***, **
TiS	0.54	2.18	**	JJ	4991.1	1675	***
FeS	0.54	2.33	***				
CuS	0.50	2.02	*	JJ	89.7	22.09	****
ZnS	0.50	1.97	**	HM, KG, KI	-85,-70.7,-69	38.4, 32, 32	**,**,**
				KM, MY	-71.3, -69.3	34.3, 32.1	**, **
PbS	0.45	1.6	*	BG, BS	-43.0, -42.96	17.6, 17.6	**, **
				IG, KY	-43.1, -42.2	19.64, 16.43	**, **
BrS	0.40	1.35	n.s	KW, KY	27.1, 21.73	9.2, 9.2	***, **
F Stat; F22, 441							

*, **, ***, **** significant at 0.06, 0.05, 0.008, 0.0002 levels of probability; Key to Table 7.6; SE=soil elements

While Jinja had the most soil tin and copper content, Entebbe and Kamwenge also had high tin content (Figure 7.5a, b). The lowest recorded soil tin content was in Mbarara (Figure 7.5a) while the least copper was in Hoima, Kayunga and Masaka (Figure 7.5b).

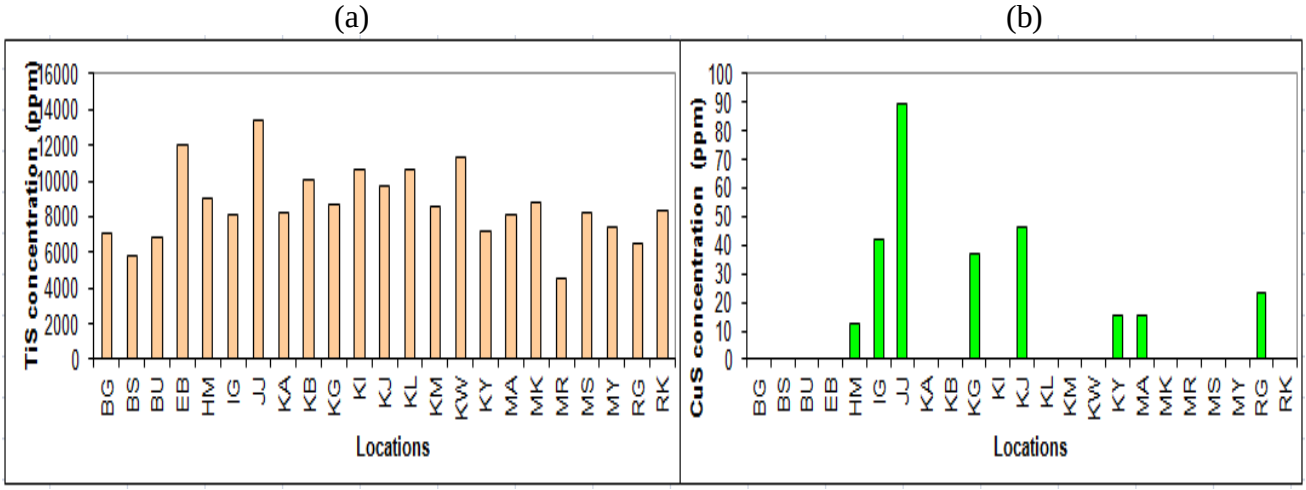


Figure 7.5 (a, b): Comparison of mean (a) soil tin and (b) copper elements in 23 locations

Differences in altitude ranges and locations for soil elements are shown in Table 7.7. Coffee leaves at elevation 1201-1300 m above sea level had significantly more tin, manganese and iron. The district of Kamwenge had significantly high amounts of leaf tin, manganese, iron and low calcium. Leaves from Kibale had significantly more leaf copper while those from Jinja had more zinc content.

Table 7.7: ANOVA of leaf elements with altitude range (m above sea level) and 23 locations

ANOVA statistic summary								
LE	R^2	d.f	v.r	p v	category	coeff	s.e	p v
TiL	0.14	5,61	1.99	*	1201-1300	1177.5	584.6	**
MnL	0.16	5,61	2.34	**	1201-1300	246.02	110.26	**
FeL	0.14	5,61	1.97	ns	1201-1300	6414.7	3185.75	**
CaL	0.45	22,44	1.62	*	KW	-6554.2	3283	**
TiL	0.48	22,44	1.87	**	KW	3532.5	815.05	****
MnL	0.48	22,44	1.85	**	KW	614.6	156.02	****
FeL	0.48	22,44	1.84	**	KW	19289	4457.5	****
CuL	0.27	22,44	0.73	ns	KI	26.48	11.50	**
ZnL	0.32	22,44	0.96	ns	JJ	38.14	14.42	**

*, **, ***, **** significant at 0.09, 0.05, 0.005, 0.0003 levels of probability.

Key to Table 7.7: LE= leaf elements; R^2=coefficient of determination; p v=probability value

The highest recorded mean of 300 ppm for manganese content was at 1201-1300 m above sea level while the rest of the elevations had about 50 ppm as given by Figure 7.6. Green bean zinc content was highest at 1401-1654 m above sea level and lowest at 1001-1200 m above sea level.

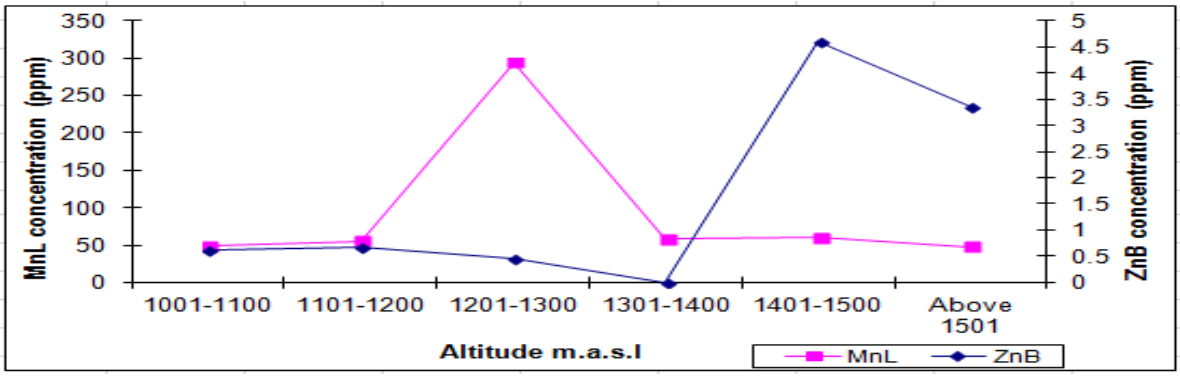


Figure 7.6: Comparing mean leaf manganese and bean zinc nutrient elements at altitude ranges

Robusta coffee trees of 11-20 years were deficient in green bean potassium, iron, copper and bromine as shown by Table 7.8. Trees of 41-50 years had low bean potassium while those of 51-60 years had also less iron. Beans at elevation 1401-1500 m above sea level had significantly low calcium while beans at height range 1001-1400 m above sea level were deficient in zinc and those at elevation 1101-1200 m above sea level had low lead content. Beans from Bushenyi, Kayunga and Masaka had significantly more calcium levels. Bean bromine was significantly high in Kyenjojo.

Table 7.8: ANOVA of green bean elements with tree age range, altitude range and 23 locations

ANOVA statistic summary								
BE	R^2	d.f	v.r	p v	category	coefficient	s.e	p v
KB	0.07	5,61	1.15	ns	11-20	-2565.6	1356	**
					41-50	-3013.3	1621.6	**
FeB	0.08	5,61	1.18	ns	11-20	-18.57	7.29	**
					51-60	-19.37	8.71	**
CuB	0.12	5,61	1.99	*	11-20	-7.9	3.37	**
BrB	0.08	5,61	1.20	ns	11-20	-3.6	1.67	**
CaB	0.16	5,61	2.28	*	1401-1500	-934.53	520	*
ZnB	0.23	5,61	3.55	**	1001-1100	-2.73	1.08	**
					1101-1200	-2.67	0.96	***
					1201-1300	-2.88	1.08	**
					1301-1400	-3.34	1.53	**
PbB	0.12	5,61	1.64	ns	1101-1200	-2.93	1.36	**
KB	0.46	5,61	1.70	*	BG	8951.11	2754.52	***
CaB	0.38	5,61	1.22	ns	BS	1716	607.29	***
					KY	1320.3	568.07	**
					MA	1217.7	607.29	**
BrB	0.30	5,61	0.85	ns	KJ	10.61	4.28	**

*, **, ***, **** significant at 0.09, 0.05, 0.008 levels of probability; Key: BE=bean elements

Mean leaf tin element values were only recorded in Kamwenge (3532.5 ppm) and Mayuge (23.9 ppm). Bugiri and Kawanda had the most potassium in green beans (Figure 7.7a) while Kamuli had the least (Figure 7.7b). Kamwenge had outstanding leaf iron content of about 19,000 ppm while the rest of locations had about 1000 ppm and below as in Figure 7.7b.

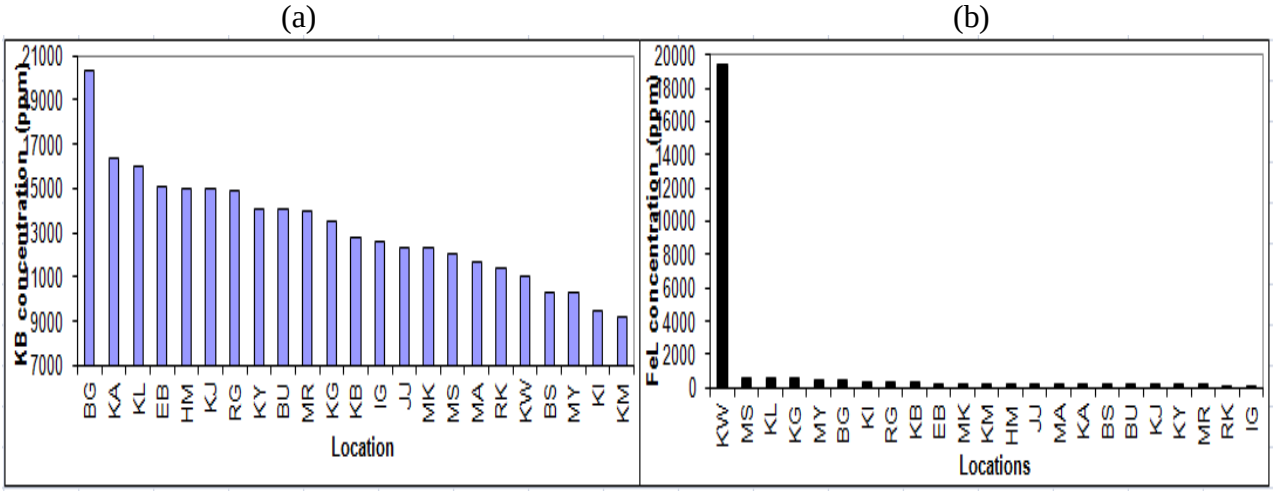


Figure 7.7: Comparison of (a) green bean potassium (b) leaf iron elements in 23 locations

7.4. Discussion

In Table 7.1, nine elements were detected in the Ugandan soils where Robusta coffee was cultivated. Of the nine elements, two were macro (potassium, calcium), four trace (copper, zinc, manganese, iron), one beneficial (bromine) and two non essential (lead, tin). Nitrogen, potassium, phosphorus, calcium, magnesium and sulphur macro elements are essential for plant growth. Of the macro elements, nitrogen, phosphorus and potassium are primary because are required in large amounts by the coffee plant for growth, development and yield (Cannell, 1985; DaMatta et al., 2007). Carbon, oxygen, hydrogen that constitute 94% of plant tissue are supplied by air and water Copper, zinc, manganese, iron, boron, molybdenum, chlorine and sodium trace elements are required in smaller amounts for the formation of vitamins, enzyme and hormones involved in almost every physiological process. A balanced application of macro and minor nutrients produces good crops and fresh cropping wood frame for the succeeding year (Iloyanomon et al., 2011).

The best soils for Robusta coffee cultivation are lava, volcanic ash, basic rocks and alluvial deposits with a high cation-exchange capacity and organic content that provides high water retention capacity and nutrient minerals to support tree growth (Wrigley, 1988; Zake et al., 1996). As from effects of various soil types on quality traits shown in Tables 4.13 and 6.6, Ugandan Robusta coffee grows in red sandy, clay or gravelly loam that may contain murrum or overlay soft laterite as reported by Wrigley, 1988.

The results indicated Robusta coffee growing areas varied in soil element occurrence and content (Table 7.6). All study sites were significantly different for soil iron content. Soil potassium was significantly low in Kabarole and Kibale while calcium was high in Bundibudyo, Iganga, Kyenjojo and Rukungiri (Table 7.6). Soil zinc nutrient was significantly low in Hoima, Kiboga, Kibale, Kamuli and Mayuge. Bugiri, Bushenyi, Iganga and Kayunga were deficient of soil lead whilst Kamwenge and Kayunga had significant amounts of soil bromine. Jinja had the most soil tin and copper content whereas Entebbe and Kamwenge had also high tin content (Figure 7.5a, b). The lowest recorded soil tin content was in Mbarara (Figure 7.5a) while the least copper was in Hoima, Kayunga and Masaka (Figure 7.5b). Nutrient element variations could be attributed to differences in genotype element uptake, farm systems along with physical and soil chemical properties that determined the composition and productivity of soil (Slagle et al., 2004). Previous plant cover and rainfall influence soil nutrient availability in crops. Excess soil minerals, such as salinization may cause salt stress and limit plant growth, though some halophytes can tolerate high salt levels and may seize some heavy metals in the vacuole while others could secrete salt from salt glands (Grusak et al., 1999).

Except for a few locations, it was apparent that soil potassium, calcium, iron, zinc, calcium, copper and bromine essential element concentration were deficient in most locations (Table 7.6), posing a health threat to the plant and coffee production constraintconstarint. Nitrogen, phosphorus and potassium elements are required in larger amounts to realize good coffee yields. Although Ugandan soils reportedly have high organic matter (6.66-17.8%), and acidity (pH 5.1-6.8) acidity that may suit Robusta cultivation, inadequate nitrogen, phosphorus, calcium and magnesium contents and exchangeable bases potentially lowers achievable yields (Zake et al., 1996). Meanwhile the results of 23 evaluated locations indicated that only Bundibudyo, Iganga, Kayunga and Rukungiri had significantly high calcium content while manganese content was below significant levels (Table 7.6). Nitrogen and phosphorus content were undetectable (Table 7.1).

Soil, leaf and green bean element interactions (Figure 7.2; 7.3; 7.4) may have influenced occurrence of low soil potassium, iron, zinc, calcium, copper and bromine contents in coffee gardens. Low soil calcium content was also observed in loam clay soil (Table 7.5). Insignificant levels of zinc and bromine content occurred in soil. Though low soil copper occurred in the presence of low soil iron (Figure 7.2), high soil copper levels were realized in Jinja (Table 7.6). By being along the equator Uganda experiences warm temperatures and high rainfall that weather or leaches basic soil cations such as calcium, magnesium and potassium leaving more stable irons ions such as Fe and Al oxides. Fe and Al oxides may increase soil acidity and adversely affect crop growth. For example, high concentration of aluminum damage roots while manganese toxicity symptoms manifest by chlorotic leaves. Since less than 20% of Ugandans use fertilizer (Zake et al., 1996), soil acidification through use of NH4+ producing fertilizers such as urea, anhydrous NH3, $(NH4)_2SO4$) is minimized. Even with no inorganic nitrogen compound fertilizers, the significantly high iron content found in most soils may induce high soil acidity (Table 7.6). The significantly

low potassium in Kabarole, Kibale (Table 7.6) and the low soil calcium content in loam clay (Table 7.5) may have resulted from effect of leaching. Coffee too is a heavy feeder depleting for instance 93% potassium over 22 years. For 6 tons of ripe coffee equivalent to 1 ton of green beans, 35 kg N, 6 kg P_2O_5, 50 kg K_2O, 4 kg CaO, 4 kg MgO, 0.3 kg Fe_2O_3 and 0.02 kg Mn_3O_4 are removed from the soil (Onzima et al, 1996).

Before amending soils, it's preferable to determine soil and plant tissue element status. Nutrients from coffee pruning and suckers can be complemented with formulated inorganic fertilizers or organic mature such as coffee pulps/hulls of which 60 kg bag contains 1 kg N; 0.60 kg P; .09 kg K and other trace elements. Excess potassium like in coffee husks positively enhances interaction of nitrogen metabolism but induces magnesium deficiency. From Table 7.1, average soil and leaf values were; potassium (8206.2, 11726.4 ppm), calcium (4939.3, 13343.9 ppm), tin (8744.6, 212.3ppm), manganese (1823.0, 96.6 ppm), iron (55111.1, 1444.1 ppm), copper (13.6, 10.0 ppm), zinc (74.4, 4.2 ppm), lead (36.8, 2.0 ppm) and bromine (12.6, 1.5 ppm) respectively. Some of the critical values for soil and foliar elements were; potassium (0.40%, 1.4%), calcium (0.89 cmol/kg, 0.37%), copper (0.3-10mg/kg, 0016-0.002%), manganese (50mg/kg, 0.005-0.01%) and iron (2-20mg/kg, 0.07-0.2%) respectively (Iloyanomon et al., 2011). Mulching with plant residue conserves soil moisture, protects soil from compaction and reduces soil acidity.

Unlike tin content that was only detected in soil and leaf, potassium, calcium, copper, zinc, manganese, iron, bromine, lead and tin were traced from soil to leaf and green bean (Table 7.1). The mean concentration of potassium increased from soil (8206.2 ppm) to 11716.4 ppm in the leaves and 12904.4 ppm in the green bean. Calcium had the lowest mean value of 4939.3 ppm in soil, but the value increased to 13343.9 ppm units in leaves and reduced to 12904.4 ppm in the green bean. Highest amounts of manganese, iron, copper, zinc, lead and bromine were respectively

recorded in soil (1823.0, 5511.1, 13.6, 74.4, 36.8, 12.6 ppm) followed by leaves (96.6, 1444.1, 10.0, 4.2, 2.0, 1.5 ppm) and lowest in beans (2.4, 48.0, 12.8, 1.0, 1.4, 1.7 ppm). Tin content was 8744.6 ppm in soil but decreased drastically to 212.3 in leaves with nothing detected in beans. Inability to detect tin in green bean or any minimal element content in leaf and green bean as indicated in Table 7.1 was possibly because it occurred in undetectable low amounts. Tin might have been required for plant growth and function but not necessarily for bean development and qualities. Single atoms or molecules are not as toxic as organic form (Grusak et al., 1999). Table 7.2 and Table 7.3 verified the presence of soil elements in the plant by their effects on green bean and leaf. Soil tin had significant effects on leaves but not green bean. Different farm systems, physical and chemical soil properties might have influenced the distribution of nutrient elemets from soil to leaf and eventually green bean.

Among elements with high concentration in leaves than green beans was calcium with 13343.9 ppm in leaves and 12,904.4 ppm in green beans (Table 7.1). Calcium constitutes cell content and cell walls, supports mitosis, root and leaf development, maintains chromosome structure, activates enzymes and translocates carbohydrates. Calcium is useful in cation-exchange reactions, detoxifies plant organic acids, and contributes to fruit ripening and quality (Browning G. (1973a, b). High respective amounts of manganese, iron, zinc and lead were recorded in leaves (96.6, 1444.1, 4.2, 2.0 ppm) than in green beans (2.4, 48.0, 1.0, 1.4 ppm). Manganese catalyses enzymatic and physiological reactions involved in chlorophyl synthesis and oxidation of carbohydrate to CO_2, H_2O and evolution of O_2. Iron is an essential component of many enzymes, metabolism of nucleic acids and synthesis and maintenance of chlorophyll. Zinc biosynthesizes Indole Acetic Acid (IAA) plant hormone, promotes nucleic acid and protein synthesis helps in the utilization of phosphorus, nitrogen and attainment of plant height. Lead which is rated among non essential elements

stimulates plant growth. Critical adult lead poisoning is 0.8 µg/L and for children 0.25 µg/L and enters the body through food, water, air and beverages (Slagle et al., 2004). Tin may have a biological function, because it positively influences grain *growth* and nitrogen fixation by *Leguminosae*.

Potassium, copper and bromine concentrations in green bean were; 1273.9, 12.8, 1.7 ppm respectively as compared to those in the leaf; 1172.6, 10, 1.5 ppm respectively (Table 7.1). Functionally, potassium helps transfer energy efficiently in chloroplasts and mitochondria when forming sugars, starches, carbohydrates, protein, lipids, ascorbate and leaf cuticles. Potassium activates enzymes involved in the metabolism and translocation of proteins and carbohydrates and enhances fruit flavor, color and increases oil content. Potassium involves in opening of stomata and osmotic balance, pH control and helps resist adverse conditions such as cold, disease and counteracts injurious effects of nitrogen as well as promoting root cell division (Gure, 2006). Meanwhile copper promotes formation of vitamin A, enzymes, proteins, chlorophyll and facilitates carbon dioxide fixation, lipid synthesis, electron transport, respiration and seed germination. Bromine may be used to synthesize alkaloids which are toxic to other organisms or have pharmacological effects for medication, recreation drugs, or in entheogenic rituals (Poisson, 1988; Yukiko Koshiro et al., 2006; Joet et al., 2010).

Except for tin, all macro or micro elements detected in soil were found in leaves and green beans, but at varying amounts (Table 7.1), implying that they had vital roles to contribute in both leaf and green bean, possibly at varying magnitudes. Among the leaf elements, the activities include photosynthesis, transpiration and gaseous exchange. The higher calcium manganese, iron, zinc and lead content found in the leaves than in the green beans may have contributed to the formation and function of biochemical reactions, plant maintenance, growth, development, reproduction and

protection. The small differences in element concentration between leaf and green bean implied that their function in both tissues was complementary (Table 7.1). According to Cannell, 1985, developing green coffee fruits have stomata and can represent 20-30% of the total photosynthetic surface on heavily bearing trees. Green coffee berries may provide 100 % of their maintenance, about 30 % of their own daily respiration needs, up to 30 % of their growth and 12 % of their total carbon requirements at the endosperm-filling stage through photosynthetic (Kumar et al., 1976; Vaast et al., 2006). Meanwhile the high potassium, copper and bromine content found in the bean than in the leaf reflected the possible major element roles towards accumulation of food compounds in the bean, enhancing bean and viability qualities (Table 7.1). It is at the bean filling development stage that the white and moist endosperm gains dry matter by attracting over 70% of photosynthetic products (De Castro et al., 2006). Mature fruits also accumulate over 95% of potassium, phosphorus and nitrogen (Cannell, 1985; DaMatta et al., 2007), justifying the high bean potassium content. Table 7.8 illustrated that the majority of trees at age range 11-20 were deficient of bean potassium, iron, copper and bromine. According to Wrigley, 1988, Robusta coffee suitably adapts and becomes highly productive after 10 years. In Table 4.10, trees of 4-30 years were significantly productive while those of 4-20 years were significantly vigorous; hence require a lot more nutrients to adequately nourish many fruits.

Trace minerals such as copper, zinc, manganese, iron, boron, molybdenum, and chlorine are essential micro nutrients. They combine with vitamins, form enzymes and involved in almost every physiological process (Gure, 2006). Leaf trace elements would be expected to support leaf processes such as photosynthesis, transpiration and gaseous exchange while bean trace elements handle mainly bean development. The Results indicated that trace elements manganese, iron and zinc had high concentrations in the leaf (96.6, 1444.1, 4.2 ppm respectively) than bean (2.4, 48.0,

1.0 ppm respectively) while copper was highest in the green bean (12.8 ppm) than leaf (10.0 ppm) (Table 7.1). Except for the varying concentrations, elements that occurred in the leaves were also present in the bean. Additionally, whether iron had the highest concentration of 1444.1 ppm in the leaf while zinc had the lowest of 4.2 ppm, functionally were complementing each other and thresholds are likely to vary in quantity. The high concentration of copper in the bean than the leaf involved processes that build proteins, carbohydrates, sugars and fat deposited in beans during development and determine seed qualities like germination. Copper also contributes to photosynthesis and respiration processes most likely in both developing green bean and leaf. Some of the specific element functions include synthesis of enzymes like Indole Acetic Acid (IAA) by zinc element.

There were other macro and micro elements undetected by X-ray analysis but which are important to leaf and green bean functions and development. Among the undetected elements were macro nitrogen, sulphur, phosphorus and magnesium and trace elements boron, chlorine, molybdenum, sodium and nickel. EDXRF low X-ray yields for light elements below sodium (z=11) in the periodic table might explain why boron (z=5) and nitrogen (z=7) were not detected among evaluated samples. Gaseous nature of some elements such as chlorine and nitrogen makes detection difficult unless in a compound form. Phosphorus, magnesium and sulphur, and molybdenum if present may have been too low to be detected or required another detection method.

Other than location, occurrence and concentrations of soil, leaf and green beans element may have been influenced by genotype and environmental factors such as elevation, soil texture (Tables 7.4; 7.5; 7.7; 7.8; Figure 7.6) consequently affecting tree growth and quality trait development. The various soil types due to the likely differences in element occurrence and content influenced bean physical characters (Table 4.13) and biochemical compounds (Table 6.6). Slagle et al., 2004

attributed soil nutrient content to the biological, geochemical cycles and antropogenic factors like deforestation, land management and crop husbandry. Significant differences in caffeine content among clay, loam, loam clay, sandy loam, stone/gravel sandy loam soil textures (Table 6.6) reflected levels of element accessibility. From the results loam clay soil texture had significantly low calcium macro element content and more copper micro element content (Table 7.5). Calcium could have leached or got depleted over years of Robusta coffee cultivation. The sticky nature of clay loam soils particularly under limited water supply could contribute to the formation of significantly less dense beans with a high roast volume increase as shown in Table 4.13. Clay soil nutrient availability in the presence of adequate moisture may sufficiently nourish coffee trees thereby producing beans with low chlorogenic acid, trigonelline and fat (Table 6.6) depending on available nutrients. Loam soils due to high fertility, produced high carbohydrate foods such as sucrose. For normal Robusta coffee growth, clay soil should be of shallow depth and freely permeable. Height influenced availability and concentration for instance soil lead significantly increased at elevation 1101-1300 (Table 7.5). When height was gained, beans were subjected to essential element deficiencies like calcium (1401-1500 m above sea level), zinc (1001-1400 m above sea level) and lead (1101-1200 m above sea level), whereas coffee leaves at elevation 1201-1300 had significantly more tin, manganese and iron (Table 7.7). At raised elevation, leaching and soil erosion may result to loss of elements as compared to gentle slopes. Plant ability to absorb and utilize available soil nutrient elements also depends on genotypes, for instance commercial varieties had high concentrations of soil tin and lead (Table 7.5). Like in all plant species, Robusta coffee genotypes adapt differently to various soil types.

CHAPTER EIGHT: PHENOTYPE AND ENVIRONMENT EFFECTS ON *Coffea canephora* QUALITY TRAITS

8.1 Introduction

The development and composition of coffee green beans that influence beverage quality are genetically and environmentally influenced by factors such as soil, climate and physiological plant growth stages (Montagnon et al., 1998; Bertrand et al., 2006). Phenotypic variability of quality traits and expression reflect effects of inherent traits and growth surrounding (Leroy et al., 2006; 2008). While Moschetto et al., 1996 found organoleptic cup differences between Guinean and Congolese genetic groups, Leroy et al., 2006 observed that variation in yield and quality traits were independent and could be improved. Coffee quality is also known to arise from precursors in green beans that include sucrose, trigonelline, chlorogenic acid, caffeine and fat (Davrieux et al., 2003; Thomas, 1935). Caffeine content in seeds is quantitatively inherited and controlled by genes with additive effects (Priolli et al., 2008). Charrier et al., 1985 attributed Robusta coffee bean size, shape, color, chemical composition and flavor to genetic constitution. Leroy et al., 2008 found high variability in *C. canephora* quality traits namely yield, bean size, chlorogenic acid content, sucrose, trigonelline content, liquor acidity and bitterness with consistent quantitative trait loci (QTLs). Ky et al., 2001a described the diversity in quality precursors such as caffeine, trigonelline, chlorogenic acids and sucrose in *C. canephora* accessions and attributed the variability for most compounds to the geographical origin of the plants within the genetic diversity groups.

Physiological stresses such as heavy crop reduce bean size as a result of carbohydrate competition among berries during bean filling (Cannell, 1985; Bertrand et al., 2004; Vaast et al., 2006). Maturation has also been reported to have a strong influence on coffee quality. Guyot et al., 1988

showed that yellow or green cherries of *Coffea canephora* Pierre ex Froehn harvested at the end of the picking season contained more mature beans (based on bean size, chemical composition and cup quality) than red cherries harvested at the beginning of the picking season. Most acid coffee is reportedly from fertile volcanic soils (Harding et al., 1987). Climate, altitude, and shade play an important role through temperature, availability of light and water during the ripening period. For instance, chlorogenic acids and fat content have been found to increase with elevation in *C. arabica* (Bertrand et al., 2006).

Several factors have assessed relationships among phenotypic traits, bean qualities and environmental factors. Yadessa et al., 2008 evaluated effects of soil properties on wild Arabica coffee in Ethiopia. Iloyanomon et al., 2011 determined *C. canephora* soil fertility of different ages. Temperatures at higher elevations or under shade are lower with a temperature fluctuation reduction of 4-5^{0}C that allows more time for coffee berries to complete bean filling yielding denser beans with intense flavour than lower altitude or under full sunlight beans (Vaast et al., 2006; DaMatta et al., 2007). Carbohydrate competition among berries during bean filling reduced bean size, weight, cup quality and vegetative growth and can be improved by decreasing fruit load (Cannell, 1985; Bertrand et al., 2004; Vaast et al., 2006). Farm management practices such as plant nutrition, shading, water supply, crop husbandry may significantly contribute to bean physical characters, biochemical composition and to human health (Lu et al., 1997; Davrieux et al., 2003). Harvest, post harvest techniques and beverage preparation procedures strongly define coffee bean and cup quality (Clarke, 1985; Joet et al., 2010).

Production of superior coffee berry beverage have also been attributed to climate conditions such as altitude, rainfall, temperature patterns, latitude, sunshine, luminosity (Guyot et al., 1966; Cannell, 1985; Decazy et al., 2003; DaMatta et al., 2007). Impacts of drought and temperature stress on

coffee physiology and production as well as ecophysiology of coffee growth and production have been evaluated (DaMatta et al., 2006; DaMatta et al., 2007). Relationships of organoleptic cup, biochemical compounds and bean physical characters (Decazy et al., 2003; De Castro et al., 2006; Vaast et al., 2006; Tessema et al., 2011) as well as with bean development stages (Ashihara et al., 1996; Koshiro et al., 2006) have been reported.

Barbosa et al., 2012 correlated environmental variables with bean chemical compounds and sensory traits revealing the influence of temperature and rainfall. Rainfall and temperature on bean development stages was identified as a major contributor to bean physical and biochemical compound development as well as tree morphology changes (De Castro et al., 2006; DaMatta et al., 2007). Water availability; particularly during the rapid fruit expansion stage often make beans larger than those that expand during the hot, dry weather (Cannell, 1985; DaMatta et al., 2007). Rains are ecologically important in determining the interval between flowering and seed maturation which for most coffee species is 8-12 months. Furthermore, the rate of fruit development is also strongly affected by air temperature (DaMatta et al., 2007). Rainfall after a dry spell induces flowering. It is therefore evident that rainfall and temperature affect tree morphology and bean physical, biochemical composition and organoleptic cup. In the Ugandan conditions, the five different bean growth stages namely; pin head, rapid cell division, endosperm, full formation and ripening that last 9 months (De Castro et al., 2006; DaMatta et al., 2006; 2007; 2008; Geromel et al., 2006; Wrigley, 1988).

Although various genotypic traits and environmental factors have been known to influence quality traits, quantitative data describing the combined effects on quality traits is lacking in Uganda. It is important to note that coffee visual appearance is an important indicator of quality, but it is not reliable. Final coffee quality depends on cup as assessed by human sensory organs and consumers

who have a specific taste according to their nationality (Leroy et al., 2006). Therefore the bean and liquor must be stable particularly for the roaster and the consumer. The objective of the study in the present chapter therefore was to determine effects of the genotype and environment on *C. canephora* quality traits. Quality attributes considered, included organoleptic cup attributes, green bean biochemical compounds, green bean physical characters and phenotypic characters. Nutrient elements, temperature and rainfall constituted environmental factors.

8.2 Materials and Methods

Data that was used to evaluate effects of Robusta coffee phenotypic and environmental factors on quality traits was derived from previous chapters as detailed below;

8.2.1 Genotype predictor and quality trait response variable data

Phenotypic character data from previous chapters that included tree morphology and green bean physical characters (Chapter 4), biochemical compounds (Chapter 5) and organoleptic cup (chapter 6) was considered as quality trait data because they are indicators of coffee quality. Because the different phenotypic characters influence each other, they were also regarded as predictor variables. Phenotypic data measurements were as in the previous chapters as follows;

a. Morphological characters included; (i) tree age in years, (ii) tree girth diameter in cm, (iii) primary internode length in cm, (iv) leaf length in cm, (v) leaf width in cm, fruits per axil in numbers (vi) tree production capacity and (vii) vigour were graded on a 1-5 scale; where 1= very few berries present in the tree; 5 = Very many berries in the tree and 1= unhealthy tree growth; 5 = healthy tree growth respectively. The different variables were measured as in Chapter 4, section 3.2

b. Green bean physical characters were; (i) roast time per gram in seconds, (ii) roasted green bean percentage weight decrease in grams, (iii) roasted green bean percentage volume increase

mm^3, seed density in grams, screen size hole 18, screen size hole 15 and above, screen size hole 15, screen size hole 12 and below). Data was measured as in Chapter 4, section 3.4

c. Green bean biochemical compounds included; (i) Dry matter, (ii) Caffeine, (iii) Chlorogenic acid, (iv) Trigonelline, (v) Sucrose, and (F) fat. Measurements were taken as in Chapter 5, section 5.3.2.1 in percentage dry matter

d. Organoleptic cup attributes included; (i) Fragrance and aroma, (ii) Flavor, (iii) Aftertaste, (iv) Salt/acid, (v) Bitter sweet, (vi) mouth feel, and (vii) balance. Rating was based on a numeric scale of 1-10 (1=least perceived and 10=strongly perceived) based on the cupper's previous experience.

8.2.2 Environment data

Environment data comprised soil leaf and green bean nutrient elements. Rainfall and temperature constituted another environmental data set. Detail of data variables and measurements was as follows;

8.2.2.1 Nutrient elements predictor data

Soil, leaf and green bean nutrient element data included (i) Potassium, (ii) calcium, (iii) tin, (iv) manganese, (v) iron, (vi) copper, (vii) zinc, (viii) lead, and (ix) bromine. Measurements were done as in Chapter 7, section 3.2.

8.2.2.2 Rainfall and temperature predictor data

In this research, rainfall and temperature data were obtained from 11 meteorological weather stations that represented study sites and shown in Table 4.1. The 9 month data was selected based on the period taken by the fruit to mature and ripen from fertilization in Ugandan conditions. By counting 9 months backwards from harvest day, rainfall and temperature data for bean development in specific areas was obtained. Consequently rainfall data variables which comprised mean rainfall

(RFM), total rainfall (RFT) and number of rainy days (RD) and two temperature variable data; mean minimum temperature (Tmin) and mean maximum temperature (Tmax) were calculated as below;

The 5 bean development stages from flowering to ripening were computed in weeks; 6-8 for pinhead stage (PHS), 10-15 for rapid cell division (RCD), 12-19 for endosperm stage (EDS), 20-29 for full formation stage (FFS) and 30-35 weeks ripening period (RPS). For each of the 5 bean development stages, the total rainfall was summed in milliliters (ml) as follows; PHS stage for 6-8 weeks (21 days), RCD stage for 10-15 weeks (35 days), EDS for 12-19 weeks (49 days), FFS for 20-29 weeks (63 days) and RPS for 30-35 weeks (35 days). Respective codes for total rainfall during specific development stages were; PHSRFT (pinhead stage total rainfall), RCDRFT (rapid cell division stage total rainfall), EDSRFT (endosperm stage total rainfall), FFSRFT (full formation stage total rainfall), RPSRFT (ripening stage total rainfall). The total rainfall for each of the bean development stage was divided by respective days to obtain mean rainfall in milliliters and coded as; PHSRFM (pinhead stage mean rainfall), RCDRFM (rapid cell division stage mean rainfall), EDSRFM (endosperm stage mean rainfall), FFSRFM (full formation stage mean rainfall), RPSRFM (ripening stage mean rainfall). The number of rainy days was also counted for each bean development stage and coded as follows; PHSRD (pinhead stage rainy days), RCDRD (rapid cell division stage rainy days), EDSRD (endosperm stage rainy days), FFSRD (full formation stage rainy days), RPSRD (ripening stage rainy days). Furthermore, meteorological records for minimum and maximum temperatures for each of the 5 bean development stages was respectively summed and divided by respective days to provide mean minimum and mean maximum temperatures in degrees centigrade (^{o}C). Mean minimum temperatures were coded as PHSTmin (pinhead stage mean minimum temperature), RCDTmin, (rapid cell division stage mean minimum temperature),

EDSTmin (endosperm stage mean minimum temperature), FFSTmin (full formation stage mean minimum temperature), RPSTmin (ripening stage mean minimum temperature). Mean maximum temperatures were also coded as; PHTmax (pinhead stage mean maximum temperature), RCDTmax (rapid cell division stage mean maximum temperature), EDSTmax (full formation stage mean maximum temperature), FFSTmax (full formation stage mean maximum temperature), RPSTmax (ripening stage mean maximum temperature).

8.2.3 Data analysis

Multivariate Analysis of Variance (MANOVA) within Palaeontological statistics 2.14 program was used to correlate genotype or environment data to identify variables with significant effects for use in multiple regressions. Palaeontological statistical program 2.14 handles more than one dependent variable and is not significantly influenced by the correlation between the independent variables. Thereafter, significant independent variables from MANOVA were collectively categorized as; (a) phenotypic quality traits (organoleptic cup, biochemical compounds, bean physical characters, tree phenotypic traits), (b) nutrient elements (soil, leaf, green bean) and (c) weather (mean rainfall, total rainfall, number of rainy days, minimum temperature, maximum temperature).

Phenotypic traits and environmental factors were regressed on quality traits to determine predictor variables with significant effects. Significantly different predictors help manage risks and maximize favorable outcomes. In this regard, categorized data sets of phenotypic traits, nutrient elements and rainfall/temperature bean development stages were independently regressed on organoleptic cup traits, bean biochemical compounds, bean physical characters and phenotypic traits with GenStat 12th edition using a hypothetical multiple linear regression relationship model; $Y=a + b_1X_1 + b_2X_2 + b_3X_3 \ldots.+ b_kX_k + I$ (where Y= response/dependent variable being predicted or explained; k=number of predictors; a= Alpha is the constant or intercept; , b_1, b_2 , b_k are Beta or

regression coefficients for X_1; and X_1 is first independent or predictor variable that is explaining the variance or linear trend in Y with a unit predictor change while other predictors are held fixed at some specified level; I= sampling error and variation in Y values from its mean; 0= mean).

8.2.4 Summary of multiple regression analyses

a. Organoleptic cup quality traits as response variables

Predictor variables included: (i). Phenotypic traits (biochemical compounds, green bean physical characters and morphological traits. (ii). Nutrient elements (soil, leaf, green bean) and (iii). Rainfall/temperature PHSRFT, RCDRFT, EDSRFT, FFSRFT, RPSRFT, PHSRFM, RCDRFM, EDSRFM, FFSRFM, RPSRFM, PHSRD, RCDRD, EDSRD, FFSRD, RPSRD, PHSTmin, RCDTmin, EDSTmin, FFSTmin, RPSTmin, PHTmax, RCDTmax, EDSTmax, FFSTmax, RPSTmax

b. Biochemical compound quality traits as response variables

Predictor variables were: (i). Phenotypic traits (green bean physical characters and morphological traits). Section (ii) and (iii) is as shown in (8.3.3.a) above.

c. Green bean physical characters as response variables

Predictor variables: (i). Tree phenotypic traits (green bean physical characters, biochemical compounds and morphological traits). Section (ii) and (iii) is as shown in (8.3.3.a) above.

d. Tree morphological traits as response variables

Predictor variables: As shown in section (a) above: (ii) and (iii).

8.3 Results

Table 8.1 shows combined significant effects of morphological traits, green bean biochemical compounds and physical characters on organoleptic cup attributes. Aftertaste, flavour and acidity were high in screen size 15 beans. Increase in caffeine reduced flavour, mouth feel, liquor acidity and cup balance. Long leaves had a positive and significant effect on mouth feel. Roast time increased cup acidity. Variability explained by phenotypic traits ranged from 3.3% (screen size 15, caffeine, leaf length) on flavor to 8.8% (screen size 15, roast time, caffeine, leaf length) on salty/acid cup.

Table 8.1: Multiple effects of Robusta coffee phenotypic traits on organoleptic cup attributes

Regression statistic summary							
Cup traits	Phenotype traits	coeff	se	v.r	F pr	t p	R^2 (%)
Aftertaste	screen size 15	0.003	0.001	2.95	**	**	4.7
Flavour	screen size 15	0.002	0.001	2.34	**	**	3.3
	caffeine	-0.16	0.07	2.34	**	**	
Mouth feel	caffeine	-0.16	0.07	2.33	**	**	3.3
	leaf length	0.02	0.01	2.33	**	*	
Salt/acid	screen size 15	0.002	0.001	4.77	***	**	8.8
	RTPG	1.7	0.65	4.77	***	***	
	caffeine	-0.19	0.07	4.77	***	***	
	leaf length	-0.01	0.009	4.77	***	**	
Fstat; F5, 191							

, * significant at 0.05 and 0.008 levels of probability

Fitted terms in the multiple regression analysis: Constant + scr15 + RTPG + Caffeine + LL + Fruits; Key to Table 8.1: s.e=standard error; d.f= degrees of freedom; F p= Fisher 95% significant levels; t p= Student t-test 95% significant levels; R^2 (%)= coefficient of determination indicates the percentage of variation in Y explained by the predictor; RTPG=roast time per gram; coeff=regression coefficient; F stat; F5, 191= Fisher statistics, 5 and 191 degrees of freedom for factor and variables respectively.

Table 8.2 shows significant multiple effects of soil, leaf and bean elements on organoleptic cup. High soil manganese increased cup fragrance and aroma. Flavour increased with high amounts of lead and iron in beans. High concentrations of bean iron enhanced aftertaste. High leaf potassium content increased cup sweetness. High bean lead content improved mouth feel. Low soil zinc content increased cup acidity. Organoleptic cup highest explained variability was 22.1% by soil manganese on fragrance/aroma while the lowest of 6.3% was by soil zinc content von salt/acid.

Table 8.2: Multiple effects of elements on Robusta coffee organoleptic cup attributes

Regression statistic summary							
Cup traits	Elements	coefficient	s.e	v.r	F pr	t pr	R^2 (%)
Faroma	MnS	1E-4	5E-5	1.95	n.s	**	22.1
Flavour	PbB	0.06	0.02	1.82	n.s	**	19.7
	FeB	0.007	0.003	1.82	n.s	**	
Aftertaste	FeB	0.008	0.004	1.66	n.s	**	16.5
Bitter/sweet	KL	3E-5	2E-5	1.24	n.s	**	6.7
Mouth feel	PbB	0.06	0.02	1.72	n.s	**	17.7
Salt acid	ZnS	-0.003	0.001	1.22	n.s	**	6.3
Fstat; F9, 21							

, * significant at 0.05 and 0.005 levels of probability; phenotypic

Fitted terms in the multiple regression analysis: Constant + MNS + PBB + CAB + FEB + ZNS + KS + KL + CAL + BRS**.** Key to Table 8.2**:** element attachment S, L, B =soil, leaf and bean elements; Mn=Manganese, Pb=Lead, Ca=Calcium; Fe=Iron, Zn=Zinc; K=Potassium, Br=Bromine

Table 8.3 shows significant effects of bean development stages rainy days and maximum temperature on organopleptic cup. Many rainy days at endosperm stage and maximum temperatures at pin head stage explained 3.2% variability for cup flavour.

Table 8.3: Multiple effects of rainfall and temperature bean development stages on Robusta coffee organoleptic cup attributes

Regression statistic summary								
Cup traits	fitted	rfall/temp	coeff	s.e	v.r	F pr	t pr	R^2 (%)
Flavour	1	EDSRD	0.02	0.01	2.69	*	**	3.2
Flavour	2	PHSTmax	0.05	0.02	2.67	*	**	3.2
Fstat; F2, 99								

, * significant at 0.05 levels of probability

Fitted terms in the multiple regression analysis: 1= Constant + RCDRD + EDSRD; 2= Constant + PHSTmax + RCDRD**;** Key to Table 8.3: coeff= coefficient; EDSRD=endosperm stage rainy days; PHSTmax= pin head stage maximum temperatures; rfall/temp=rainfall and temperature bean development stages

Table 8.4 shows significant effects of bean physical characters and morphological traits on biochemical compounds. High dry matter beans were of screen size 18, from trees with large tree girth and many stems. Caffeine and chlorogenic acid concentration increased with prolonged roasting and from vigorous tree beans. Chlorogenic acid content was high in less dense beans. Trigonelline content was reduced in less dense beans and beans from trees with a small tree girth (young). High sucrose beans were from trees with small girth (young), many stems and short leaves. Fat was high in less dense and broad leaf tree beans. Except for 3.8% caffeine variability explained by prolonged roast time and tree vigour, the rest of the green bean physical characters and morphological traits explained 13.5% to 15.7% green bean biochemical compound variability.

Table 8.4: Multiple effects of Robusta coffee phenotypic traits on green bean biochemical compounds

Regression statistic summary							
Biocpds	Mxtrs	coefficient	se	v.r	F pr	t p	R^2 (%)
Dry matter	screen size 18	0.01	0.004	5.0	***	***	14.0
	girth	0.006	0.002	5.0	***	***	
	stems	0.06	0.02	5.0	***	***	
Caffeine	RTPG	2.0	0.72	1.98	**	***	3.8
	vigour	0.09	0.05	1.99	**	**	
Chlorogenic	seed density	-0.07	0.03	5.55	***	***	15.7
acid	RTPG	2.51	1.45	5.55	***	*	
	vigour	0.16	-0.09	5.55	***	*	
Trigonelline	seed density	-0.004	0.002	5.17	***	*	14.5
	girth	-0.4E	0.2E4	5.17	***	***	
Sucrose	girth	-0.005	0.002	4.8	***	**	13.5
	Stems	0.05	0.02	4.8	***	***	
	LL	-0.10	0.03	4.8	***	***	
Fat	SDD	-0.09	0.03	5.26	***	***	14.8
	LW	-0.16	0.07	5.26	***	**	
Fstat; F8, 188							

, * significant at 0.05 and 0.005 levels of probability.

Fitted terms in the multiple regression analysis: Constant + scr18 + SDD + RTPG + Girth + Stems + Vigour + LL + LW; Key to Table 8.4**:** RTPG=roast time per gram; scr18=screen size 18; Key to Table 8.4: Mxtrs=morphological characters

Significant multi effects of soil and bean elements on biochemical compounds are shown in Table 8.5. Variability of 8.4% in caffeine was explained by low bean bromine and high bean zinc. Trigonelline content variability of 31.2% was explained by low soil lead and high bean bromine.

Table 8.5: Multiple effects of elements on Robusta coffee green bean biochemical compounds

Regression statistic summary							
Biocpds	Element	coefficient	s.e	v.r	F pr	t pr	R^2 (%)
Caffeine	BrB	-0.04	0.02	1.34	n.s	**	8.4
	ZnB	0.12	0.05	1.34	n.s	**	8.4
Trigonelline	PbS	-0.001	5E-42	2.70	**	**	31.2
	BrB	0.006	0.003	2.70	**	**	
Fstat; F8, 22							

** Significant at 0.05 levels of probability

Fitted terms in the multiple regression analysis: Constant + PBS + CAS + FEB + CUS + MNS + MNL + BRB + ZNB; Key to Table 8.5: Biocpd=biochemical compounds; element attachment S, B =soil and bean elements

Significant effects of rainfall and temperature bean development stages on green bean biochemical compounds are shown Table 8.6. Dry matter was reduced at low minimum temperature during rapid cell division and low maximum temperatures at full bean formation. Many rainy days at pin head stage reduced caffeine content. High chlorogenic acid was deposited at low maximum (pin head) and high maximum temperature (endosperm), many rainy days (endosperm), high total rainfall (pin head), high mean rainfall (full formation) and low total rainfall at full formation. High maximum temperatures increased trigonelline nitrogenous base content at pin head and rapid cell division stages and reduced during endosperm and ripening period (Table 8.6). High minimum temperatures at endosperm stage and many rainy days at pinhead increased trigonelline content. High mean rainfall at pin head and rapid cell division stages decreased trigonelline production. At high maximum temperatures, fat production increased at pin head and reduced at endosperm and ripening period. Many rainy days increased fat content at pin head, full formation and ripening period. Low mean rainfall reduced fat content at pinhead stage. Except for a few rainy days at pin head stage that explained 3.7% variability in caffeine, other rainfall and temperature development stages explained a tune of 14.5% to 35.5%.

Table 8.6: Multiple effects of rainfall and temperature bean development stages on Robusta coffee green bean biochemical compounds

Regression statistic summary									
Biocpds	fitted	rfall/temp	coeff	s.e	d.f	v.r	F pr	t pr	R^2 (%)
Dry matter	1	RCDTmin	-0.13	0.04	2,99	9.57	***	***	14.5
Dry matter	1	FFSTmax	-0.43	0.14	2,99	9.57	***	***	
Caffeine	2	PHSRD	-0.02	7E-3	2,98	2.90	*	**	3.7
CGA	3	PHSTmax	-0.20	0.01	6,94	4.02	***	**	
CGA	3	EDSTmax	0.87	0.27	6,94	4.02	***	***	
CGA	3	EDSRD	0.09	0.03	6,94	4.02	***	***	15.3
CGA	3	PHSRFT	3E-3	9E-4	6,94	4.02	***	***	
CGA	3	FFSRFM	30.5	13.5	6,94	4.02	***	**	
CGA	3	FFSRFT	-2.77	1.23	6,94	4.02	***	**	
Trigonelline	4	EDSTmin	0.05	0.02	8,92	4.69	***	***	
Trigonelline	4	PHDTmax	0.25	0.08	8,92	4.69	***	***	
Trigonelline	4	RCDTmax	0.29	0.10	8,92	4.69	***	***	
Trigonelline	4	EDSTmax	-0.89	0.29	8,92	4.69	***	***	22.8
Trigonelline	4	RPSTmax	-2E-4	6E-5	8,92	4.69	***	***	
Trigonelline	4	PHSRD	0.05	0.02	8,92	4.69	***	***	
Trigonelline	4	PHSRFM	-0.01	5E-3	8,92	4.69	***	**	
Trigonelline	4	RCDRFM	-3E-3	1E-3	8,92	4.69	***	***	
Sucrose	5	RCDRD	-0.16	0.02	3,97	19.37	***	***	35.50
Sucrose	5	RPSRD	0.04	0.02	3,97	19.37	***	**	
Fat	6	PHSTmax	2.02	0.64	8,92	4.34	***	***	
Fat	6	EDSTmax	-5.96	1.95	8,92	4.34	***	***	
Fat	6	RPSTmax	-2E-3	6E-4	8,92	4.34	***	***	
Fat	6	PHSRD	0.28	0.08	8,92	4.34	***	***	21.10
Fat	6	FFSRD	0.11	0.06	8,92	4.34	***	**	
Fat	6	RPSRD	0.20	0.1	8,92	4.34	***	**	
Fat	6	PHSRFM	-0.13	0.06	8,92	4.34	***	***	

*, **, *** significant at 0.06, 0.05 and 0.009 levels of probability

Fitted terms in the multiple regression analysis: 1= Constant + RCDTmin + FFSTmax; 2= Constant + FFSTmax + PHSRD; 3= Constant + PHSTmax + EDSTmax + EDSRD + PHSRFT + FFSRFM + FFSRFT; 4= Constant + EDSTmin + PHSTmax + RCDTmax + EDSTmax + RPSTmax + PHSRD + PHSRFM + RCDRFM; 5= Constant + RPSTmax + RCDRD + RPSRD; 6= Constant + PHSTmax + EDSTmax + RPSTmax + PHSRD + FFSRD + RPSRD + PHSRFM + RCDRFM.

Key to Table 8.6: Development stages; PHS=pin head stage, RCD=rapid cell division, EDS=endosperm stage, FFS=full formation stage, RPS=ripening period; Attachments to the developments stages: RFM=mean rainfall; RFT=total rainfall, RD=rainy days; Tmin=minimum temperature; Tmax=maximum temperature; coeff=regression coefficient

Significant effects of morphological traits and biochemical compounds on green bean physical characters are shown in Table 8.7. Beans that roasted longer were from trees with a large tree girth (old) and low vigour. Young trees explained 5.2% variability for high roast bean volume percentage increase. Low amounts of chlorogenic acid and trigonelline, narrow and long leaves and increased bean density. Beans with low caffeine and trigonelline contents, trees with high dry matter, productivity, narrow and long leaves had mainly screen size 18 beans. Screen size 15 beans had high sucrose, low dry matter, caffeine and trigonelline content. Screen size 15 beans were from trees with many stems, long internodes and many fruits. Screen size 15 and above beans had high sucrose content and low chlorogenic acid and trigonelline concentrations. Screen size 15 and above beans were from trees with many fruits, long and narrow leaves. Screen size 12 and below beans had high chlorogenic acid and trigonelline content, few fruits, short and broad leaves. Bean physical character variation of 8.0-29.25% was explained by morphological traits and biochemical compounds.

Table 8.7: Multiple effects of Robusta coffee phenotypic traits on green bean physical characters

Regression statistic summary							
Bean traits	Phenotype traits	coefficient	se	v.r	F pr	t p	R^2 (%)
RTPG	girth	3E-4	1E-4	3.18	***	***	13.5
	vigour	-0.01	7E-4	3.18	***	**	
RB	age	-0.21	0.10	1.77	**	**	5.2
SDD	chlorogenic acid	-1.40	0.38	6.76	***	***	
	trigonelline	-11.61	3.40	6.76	***	***	29.2
	leaf width	-0.80	0.23	6.76	***	***	
	leaf length	0.49	0.11	6.76	***	***	
Scr18	dry matter	6.79	2.24	6.02	***	***	
	caffeine	-13.56	6.01	6.02	***	**	
	trigonelline	-79.1	25.5	6.02	***	***	26.4
	leaf width	-4.85	1.72	6.02	***	***	
	production capacity	6.98	3.20	6.02	***	**	
	leaf length	3.18	0.82	6.02	***	***	
Scr15	dry matter	-7.41	2.43	2.21	***	***	
	Chlorogenic acid	-7.32	3.05	2.21	***	**	
	Sucrose	6.40	2.59	2.21	***	**	8.0
	stems	1.0	0.45	2.21	***	**	
	internode length	3.35	1.47	2.21	***	**	
	fruits	1.02	0.40	2.21	***	**	
Scr≥15	chlorogenic acid	-10.5	2.86	5.17	***	***	
	trigonelline	-71.4	25.9	5.17	***	***	
	leaf width	-6.66	1.75	5.17	***	***	22.9
	leaf length	3.17	0.83	5.17	***	***	
	fruits	0.86	0.38	5.17	***	**	
Scr≤12	chlorogenic acid	10.5	2.86	5.17	***	***	
	trigonelline	71.4	25.9	5.17	***	***	
	leaf width	6.66	1.75	5.17	***	***	22.9
	leaf length	-3.17	0.83	5.17	***	***	
	Fruits	-0.86	0.38	5.17	***	**	
Fstat; 14, 182							

, * significant at 0.05 and 0.009 levels of probability

Fitted terms in the multiple regression analysis: Constant + dry matter + caffeine + CGA + sucrose + trigonelline + girth + vigour + stems + age + LW + PC + LL + IL + fruits

Key to Table 8.7: Scr=screen size

Table 8.8 shows significant effects of elements on green bean physical characters. Leaf bromine explained 50.9% variability in green bean weight loss. High bean potassium and bean zinc contents, low amounts of leaf manganese, bean copper and bean lead produced beans with a high roast volume increase. Screen size 15 beans were produced in low concentrations of soil lead, leaf potassium and bean zinc. Soil, leaf and green bean elements explained 24.3-50.9% of the bean physical character variability.

Table 8.8: Multiple effects of nutrient element on Robusta coffee green bean physical characters

Regression statistic summary							
Bean traits	elements	coefficient	s.e	v.r	F pr	t pr	R^2 (%)
B	BrL	4.78	1.11	3.39	**	***	50.9
RB	MnL	-0.11	0.05	3.19	**	**	
	KB	0.002	0.001	3.19	**	**	
	CuB	-1.11	0.37	3.19	**	***	48.7
	ZnB	6.52	2.05	3.19	**	***	
	PbB	-1.90	0.82	3.19	**	**	
Scr15	PbS	-0.54	0.19	1.74	ns	**	
	KL	-0.002	0.001	1.74	ns	**	24.3
	ZnB	-9.31	3.26	1.74	ns	**	
Fstat; F13, 17							

*, **, *** significant at 0.05 and 0.008 levels of probability; Fitted terms in the multiple regression analysis: Constant + ZNS + CAS + MNS + CUS + PBS + KL + MNL + BRL + KB + CAB + CUB + ZNB + PBB;Key to Table 8.8: element attachment S, L, B =soil, leaf and bean elements

Effect of bean development stages temperature and rainfall on bean physical characters is shown on Table 8.9. Beans that roasted longer were influenced by high mean rainfall (full formation, pin head), low (full formation) and high total rainfall (pin head) at a maximum variance of 25.3%. High roast bean volume was obtained with low mean rainfall (endosperm, pin head, rapid cell division) and high (full formation), high total rainfall (pin head, rapid cell division) and low total rainfall (full formation). Beans became denser with low minimum temperature variance of 8% at endosperm stage. Screen size 18 beans developed with many rainy days at rapid cell division.

Meanwhile screen size 15 beans developed at high mean rainfall (endosperm, pin head, rapid cell division), low total rainfall (pin head, rapid cell division) and a few rainy days at endosperm stage. Many rainy days at full formation and low total rainfall at pin head stage produced beans of screen size 15 and above. Screen size 12 beans occurred at high total and mean rainfall (pin head stage) and few rainy days at full formation stage. Bean physical character variability explained by rainfall and temperature bean development stages was 5.1-25.3%.

Table 8.9: Multiple effects of temperature and rainfall on Robusta coffee green bean physical characters

Regression statistic summary									
Bean traits	fitted	rfall/temp	coeff	s.e	d.f	v.r	F pr	t pr	R^2 (%)
RTPG	1	FFSRFM	1.65	0.38	3,97	12.31	***	***	25.3
RTPG	1	FFSRFT	-0.15	0.04	3,97	12.31	***	***	25.3
RTPG	1	PHSRFT	1E-04	5E-05	3,97	12.31	***	**	25.3
RTPG	2	PHSRFM	8E-04	4E-04	3,97	12.33	***	**	25.3
RB	3	EDSRFM	-1.88	0.87	6,94	2.37	**	**	
RB	3	FFSRFM	1.03	0.38	6,94	2.37	**	***	
RB	3	PHSRFM	-164.3	69.8	6,94	2.37	**	**	7.6
RB	3	PHSRFT	20.65	8.76	6,94	2.37	**	**	
RB	3	RCDRFM	-797	292	6,94	2.37	**	***	
RB	3	RCDRFT	159.6	58.6	6,94	2.37	**	***	
RB	4	FFSRFT	-82.5	32.5	4.96	2.33	*	**	5.1
SDD	5	EDSTmin	0.35	0.14	5.95	5.36	***	**	8.0
Scr18	6	RCDRD	1.54	0.73	3,97	3.17	**	**	6.1
Scr15	7	EDSRFM	2.63	1.19	6,94	3.0	**	**	
Scr15	7	PHSRFM	246.8	96.5	6,94	3.0	**	**	
Scr15	7	PHSRFT	-31.0	12.1	6,94	3.0	**	**	10.7
Scr15	7	RCDRFM	944	379	6,94	3.0	**	**	
Scr15	7	RCDRFT	-188.9	75.9	6,94	3.0	**	**	
Scr15	7	EDSRD	-2.90	0.93	6,94	3.0	**	***	
scr≥15	8	FFSRD	0.77	0.36	3,97	4.53	***	**	9.6
scr≥15	9	PHSRFT	-0.09	0.03	2,98	5.95	***	***	9.0
Scr12	10	PHSRFT	0.09	0.03	2,98	5.95	***	***	
Scr12	10	FFSRD	-0.60	0.34	2,98	5.95	***	**	9.0
Scr12	11	PHSRFM	0.69	0.24	2,98	5.90	***	***	

, * significant at 0.05 and 0.009 levels of probability; Fitted terms in the multiple regression analysis: 1= Constant + FFSRFM + FFSRFT + PHSRFT; 2= Constant + FFSRFM + FFSRFT + PHSRFM; 3= Constant + EDSRFM + FFSRFM + PHSRFM + PHSRFT + RCDRFM + RCDRFT; 4= Constant + EDSRFM + FFSRFM + FFSRFT + PHSRFT; 5= Constant + EDSTmin + RCDRD; 6=Constant + EDSTmin + RCDRD + RCDTmin; 7= Constant + EDSRFM + PHSRFM + PHSRFT + RCDRFM + RCDRFT + EDSRD; 8= Constant + PHSRFM + PHSRFT + FFSRD; 9= Constant + PHSRFT + FFSRD; 10= Constant + PHSRFT + FFSRD; 11= Constant + PHSRFM + FFSRD

Key to Table 8.9: coeff=regression coefficient; rfall/temp=rainfall and temperature bean development stages

Significant effects of soil, leaf and green bean nutrient elements on tree morphological characters are shown in Table 8.10. High soil copper increased tree girth. Tree vigour increased with high soil

zinc content. Increase in soil copper increased the production many stems. Low soil iron and bean magnesium contents produced long internodes. A reduction in soil and leaf copper generated long and broad leaves. Elements explained 13.9-44.4% of morphological trait variability.

Table 8.10: Multiple effects of nutrient elements on Robusta coffee tree phenotypic growth parameters

Regression statistic summary							
Phenotype traits	elements	coefficient	s.e	v.r	F pr	t pr	R^2 (%)
Girth	CuS	0.66	0.25	2.03	*	**	23.7
Vigour	ZnS	0.007	0.002	1.81	n.s	***	19.5
Stems	CuS	0.06	0.03	1.72	n.s	**	17.8
IL	FeS	-4E-5	1E-5	3.66	***	***	44.4
	MnB	-0.08	0.04	3.66	***	**	
LL	CuS	-0.04	0.02	1.75	n.s	**	18.3
	CuL	-0.06	0.03	1.75	n.s	**	
LW	CuS	-0.02	0.008	1.54	n.s	**	13.9
	CuL	-0.04	0.02	1.54	n.s	**	
Fstat; F9, 21							

*, **, *** significant at 0.09, 0.05 and 0.009 levels of probability; Fitted terms in the multiple regression analysis: Constant + TIS + ZNS + CUS + CAS + FES + CUL + MNL + CUB + MNB; Key to Table 8.10: element attachment S, L, B =soil, leaf and bean elements

Significant effects of temperature and rainfall bean development stages on phenotypic growth parameters are shown in Table 8.11. Many rainy days at pin head stage enhanced tree production capacity. Trees were vigorous at low mean rainfall (full formation), high total rainfall (full formation) and many rainy days (pin head). Few rainy days generated many stems at endosperm stage. High maximum temperature during ripening period influenced inter node length elongation. Long leaves were obtained at high minimum temperature (full formation), many rainy days and low minimum temperature at rapid cell division. Broad leaves developed at low temperature during pin

head stage as well as many rainy days at rapid cell division. Rainfall and temperature bean development stages accounted for 3.12-18% morphological trait variability.

Table 8.11: Multiple effects of bean development stages temperature and rainfall on Robusta coffee tree phenotypic growth parameters

Regression statistic summary									
Pheno	fitted	rfall/temp	coefficient	s.e	d.f	v.r	F pr	t pr	R^2 (%)
PC	1	PHSRD	0.16	0.09	4,96	4.49	***	***	12.2
Vigour	2	FFSRFM	-19.2	6.20	3,96	4.80	***	***	
Vigour	2	FFSRFT	1.75	0.56	3,96	4.80	***	***	3.12
Vigour	2	PHSRD	0.04	0.01	3,96	4.80	***	***	
Stems	3	EDSRD	-0.27	0.09	2,98	9.03	***	***	13.8
IL	4	RPSTmax	3E-4	1E-4	2,98	2.84	*	**	3.6
LL	5	FFSTmin	1.66	0.62	3,97	8.30	***	***	
LL	5	RCDRD	0.26	0.08	3,97	8.30	***	***	18.0
LL	5	RCDTmin	-1.25	0.59	3,97	8.30	***	**	
LW	6	PHSTmin	-1.24	0.58	7,93	2.17	**	**	7.6
LW	6	RCDRD	0.24	0.10	7,93	2.17	**	**	

*, **, *** significant at 0.08, 0.05 and 0.004 levels of probability

Fitted terms in the multiple regression analysis: 1= Constant + EDSTmax + EDSTmin + FFSTmin + PHSRD; 2= Constant + FFSRFM + FFSRFT + PHSRD; 3= Constant + EDSRD + RPSTmax; 4= Constant + EDSRFM + RPSTmax; 5= Constant + FFSTmin + RCDRD + RCDTmin; 6= Constant + RCDRFM + RCDRFT + EDSRD + EDSTmin + FFSTmin + PHSTmin + RCDRD

8.4 Discussion

Table 8.1 to Table 11, indicated that many genotypic and environmental factors influenced quality traits. Phenotypic traits that comprised of tree morphological characters, bean physical characters and biochemical compounds and environmental factors that included nutrient elements, rainfall and temperature explained 2.0%-51% of quality trait variability. Inability to explain 100% variability implied other factors in addition to what the study considered significantly influenced the quality traits. The regression coefficient values reflected the relevant contribution of each factor at different magnitudes to the overall quality trait expression, however small. The high number of evaluated accessions could have contributed to the low regression coefficient values. Inability to

generate significant effects from all genotype and environment factors implied some factors had more influence than others. Other investigators have also expressed difficulty in assessing quality traits because of the many hundreds of substances, some of which get transformed during processing (De Maria et al., 1996; Ginz et al., 2000; Clifford et al., 1985).

Regulating bean roasting and brewing temperature determine coffee flavours such as increased cup acidity shown in Table 8.1. Physiochemical parameters are influenced by genetically diverse *C. canephora* cultivars (Figure 5.4; 5.5), crop husbandry, shade, light, actual tree yield, location of bean in the primary (Charrier et al., 1985; Vaast et al., 2006). Prolonged bean development increases bean size and biochemical compound deposits while reduced crop yield minimizes competition for nutrients among berries (Vaast et al., 2006). Rainfall and sunshine distribution also induce flowering (De Castro et al., 2006). Silva et al., 2005 reported that elevation, but not soil water availability influenced bean biochemical composition. In Figures 5.4 and 5.5, biochemical compounds were genetically variable while in Table 8.4, morphological and green bean physical characters explained 13.5% to 15.7% of green bean biochemical compound variability. Meanwhile bean bromine and bean zinc explained 8.4% variability in caffeine while 31.2% variability in trigonelline content was explained by soil lead and bean bromine. Dry processing enhances coffee bitterness (Clarke, 1985). Harvest and post harvest procedures that reduce defects and maintain moisture content at 12% improve coffee quality. These results therefore indicate that Robusta coffee quality trait variability is explained by a diversity of factors that include genetic, phenotypic traits, tree physiology and environment.

In Table 8.1, 3.3%-8.8% of organoleptic cup variability was explained by morphological traits, green bean physical and biochemical compounds. Soil, leaf and green bean explained 6.3%-22.1% of cup attributes (Table 8.2). Whereas many rainy days at endosperm stage explained 3.2% of cup

attribute variability while high maximum temperatures at pin head stage explained 3.2% (Table 8.3). Unevaluated factors therefore explained up to 96.8% of organoleptic cup trait variability. In Table 8.2 leaf potassium, soil manganese and zinc, bean lead and iron element contents explained most of the organoleptic cup variability (6.3-22.1%) possibly because essential and non essential elements used to biosynthesize biochemical compounds influence the perception in the mouth either directly or indirectly through interaction. For example, high bean potassium and calcium produces harsh, bitter and brackish aftertaste (high saltiness and displeasing aroma) in Robusta coffee (Clifford et al., 1985; Wintgens, 2004). The sweet cup from high leaf potassium (Table 8.2) could have resulted from one of potassium functions of activating enzymes involved in the metabolism and translocation of proteins, carbohydrates, enhancing fruit flavor, color and oil content. (Gure, 2006). Low potassium levels are savory with low saltiness and pleasing aroma. Excessive use of potassium rich pennisetum purpurem as mulch provides much potassium to the soil. According to Wallis, 2005, magnesium deficiency associated with high potassium (cationic imbalance) reduced coffee quality (Wintgens, 2004). Too much nitrogen fertilizer may increase bean caffeine and a bitter beverage while low levels of both potassium and caffeine make the coffee brew coarse and harsh.

The results showed that soil manganese explained 22.1% of increased fragrance/aroma variability while low soil zinc content explained 6.3% of heightened salt/acid variability (Table 8.2). The 19.7% increased flavour variability was independently explained by high bean lead and bean iron contents. Meanwhile, high concentrations of bean iron explained 16.5% of the enhanced aftertaste. High bean lead content improved mouth feel as shown in Table 8.2. According to Yadessa et al., 2008, Ethiopian soils with a high pH, magnesium, manganese zinc had a better aroma, although zinc produced poor organoleptic cup quality in another site. Yadessa et al., 2008 further found

better coffee quality in soils rich in phosphorus, potassium, clay and silt but not sand content, with site specific effect of micro nutrients on coffee quality.

Phenotypic traits that significantly explained organoleptic cup variability included screen size 15, roast time, caffeine and leaf length (Table 8.1). The significantly good aftertaste, flavour and cup acidity in screen size 15 beans (Table 8.1) could have resulted from low caffeine and dry matter (Table 8.7). In Table 8.1, caffeine was found to lower flavour, mouthfeel and salt/acid. Low caffeine and chlorogenic acid content in screen size 15 beans (Table 8.7) possibly minimized the bitter cup taste. Undesirable bitter liquor correlates with dissolved coffee solids (Griffin, 1999; Wang, 2012). At medium roast, coffee has less soluble solids, a higher acid content, and a potent aroma as compared to the dark roast. The high cup acidity realized from screen size 15 beans was possibly developed from skillful roasting as per genotype and bean density while high sucrose content contributed to the high rating of flavour and aftertaste (Table 8.1). Roast time increased cup acidity (Table 8.1). Light roasted beans have more acidity, aroma and sweetness than dark roast (Wang, 2012). The three main groups of roasted coffee bean acids include; aliphatic chlorogenic, alicyclic carboxylic and phenolic acids. Phosphoric acid may constitute compounds that influence coffee acidity but not directly in the liquor (Maier, 1987; Griffin, 1999). Citric acid, malic acid, and acetic acid may be the major compounds that contribute to cup acidity. However, highly complex buffering effects and the wide distribution of salts and acids present in coffee make it difficult to predict the exact mechanism and crucial acids in perceived in coffee acidity.

High flavour in screen size 15 beans could have arisen from increased bean sucrose. In Table 5.1, Ugandan sucrose content was high with a wide variability of 2.48-7.34 that exceeded 4.05–7.05 reported by Ky et al., 2001a and 5.0-7.0 for Wintgens, 2004 (Table 5.2). Sucrose degrades rapidly during roasting, forming a wide range of compounds such as aliphatic acids, anhydro-sugars and

other compounds like glyoxal involved in coffee flavor, either as the volatile aroma compounds, or as non-volatile taste compounds (Clifford, 1985; De Maria et al., 1994; Tressl et al., 1998; Ginz et al., 2000; Campa et al., 2004). In coffee and many other plants, sucrose is essential for the control of sink-source mechanisms (Geromel et al., 2006) and is one of the main compounds in coffee beans, that varies from 5% to 9.5% of dry matter (dm) in *Coffea arabica* and from 4% to 7% of dm in *Coffea canephora* (Tessema, et al., 2011). Even after considerable degrading by roasting, 0.4-2.8% dry matter content contributed to Arabica coffee sweetness. Amino acids also produce aromatics during the Maillard reactions that diminish after roasting for 5 minutes at 220°C (Maier, 1987). Flavour variation by different bean size biochemical compounds offer opportunities for diverse markets and price offers. The phenotypic description of trees with reduced girth, many stems and short leaves with significantly high sucrose were bound to be the "nganda" types (Table 4.3; Table 8.4; Figure 6.8b).

Though less dense, beans from broad leaf trees had significantly more fat (Table 8.4). Ugandan Robusta coffee had excessive fat content of range 10.44-15.94 as compared to that reported by Wintgens, 2004 (7.0-11.0) but comparable to Arabica coffee (13.0-17.0) (Table 5.2). Bean fat content was associated with flavour and overall assessment of (Tessema et al., 2011). Larcher, 2005 and Barbosa et al., 2005 reported positive association between fat and sensorial quality attributes like aroma, body, acidity, flavor, aromatic intensity and preference. Decazy et al., 2003 found that fat content was associated with acidity and beverage preference. Fat fixes flavour compounds formed during roasting (Davrieux et al., 2003; Charrier et al., 1985). The high sucrose and excessively high fat content observed in evaluated beans (Table 5.2) may explain why organoleptic cup rating for all Ugandan genotypes was of average to fine quality (Table 6.4). According to Davrieux et al., 2003, sucrose and trigonelline contents offer a better aroma. The high trigonelline

contents in screen size 12 and below beans coupled with high fat content may enhance organoleptic cup despite the low bean density, high levels of chlorogenic acid, trigonelline.

Bean size was reduced at 1400-1500 m above .sea level (Table 4.6) and had a weak bean aftertaste (Table 6.7). Coffee beans from height 1001-1100 m above sea level and 1201-1400 m above sea level had the most cup acidity while the best cup balance was being found at height 1300-1400 m above sea level (Table 6.7). At high altitudes, beans developed low acidity possibly because at high elevation leaves often get distorted and discolored, a condition referred to as "hot and cold effect". As a consequence of poor plant growth, yield and flavour is reduced and improper bean structure developed. Many rainy days at endosperm stage and maximum temperatures at pin head stage explained 3.2% of the variability in cup flavour (Table 7.3). Moist soils ease access of nutrients by roots that transport them to green leaves and beans for synthesis and bean deposition of biochemical compounds that increase flavour at endosperm stage. Good bean sensory qualities require high altitudes and well distributed annual rainfall of less than 1500 mm (Decazy et al., 2003). Moisture is required by soil micro organisms to make certain nutrients available for the plant, to take up nutrients from soil by roots for Cation Exchange Capacity (CEC). Decomposition by bacteria, negatively charged soil and organic particles generate acids in moisture, promoting weathering of rock to release of K^{+}, Mg^{2+} and Mn^{2+} (Zake et al., 1996). Detected flavour at maximum temperatures during pin head stage could have resulted from accumulated sugars prior to rapid cell division stage.

Green bean biochemical compound variability of 3.8%-15.7% range was explained by bean physical characters and morphological traits (Table 8.4). While nutrient elements explained 8.4%-31.2% of the biochemical compound variability (Table 8.5), rainfall and temperature at different bean development stages (Table 8.6) explained 3.7-35.5% of the variability. About 64-96% of the

biochemical variability was therefore not accounted for by the predictors studied here. These findings indicate rainfall and temperature bean development stages contributed more to the deposition of green bean biochemical compounds, followed by soil lead, bean bromine and bean zinc elements and least by phenotypic traits (morphological and bean physical characters). According to DaMatta et al., 2006 drought and unfavourable temperatures are the major climatic limitations for coffee production that affect tree physiology and reproduction. Low production of dry matter at low temperatures during rapid cell division as shown by Table 8.6 arose because cells at that stage were designed to divide to form swollen locules containing ovaries other than storage.

Deposition of dry matter was minimal at high maximum temperatures during bean full formation (Table 8.6). Net photosynthesis in coffee ceases almost completely at 5-10 ºC, while above 24ºC a drop of 10 % in production of dry matter would occur for each 1ºC rise, such that dry matter accumulation approaches zero at 34ºC (DaMatta et al., 2006). Dry matter accumulation in crops also depend on the nutrient element availability, leaf area index, canopy structure, photosynthetic rate per unit leaf area and the strength of the metabolic sinks to attract assimilates. Optimum light intensity for maximum photosynthetic efficiency in coffee ranges from 900 to 1300 Einsteins. Carbohydrate photosynthetic contribution to the plant is reduced in poorly developing green beans (Kumar et al., 1976; Vaast et al., 2006). Low caffeine production at pin head stage with many rainy days as indicate in Table 9.6 conformed to that reported by other authors (Ashihara et al., 1996; Koshiro et al., 2006). Wet conditions may have reduced tree moisture stress that would have induced much caffeine production.

In Table 8.6, mean maximum temperatures reduced chlorogenic acid during pin head stage and increased it at the endosperm stage. Low total rainfall reduced chlorogenic acid at full formation stage and increased the acid at high total rainfall (pin head), many rainy days (endosperm stage) and

high mean rainfall (full formation). According to Bertrand et al., 2003, most chlorogenic acid was produced by young fruits during endosperm stage while Joet et al., 2010 associated high chlorogenic acid and isomer biosynthesis with mean temperature. Whereas Ashihara et al., 1996, indicated chlorogenic acid production was influenced by the biogeographic origin of the plant material. A few rainy days at rapid cell division and many rainy days at ripening period were required for sucrose deposition as shown in Table 8.6. High hexose to sucrose ratios occur at early perisperm stages and endosperm development characterized by intensive cell division in young tissues (Geromel et al., 2008: 2006). After endosperm stage, high to low hexose to sucrose content is expected as the fruit changes from expansion to protein and sucrose storage (Rogers et al., 1999a; Geromel et al., 2006). At ripe cherry harvest, both soluble reducing sugars and sucrose buildup from carbohydrate-degrading enzymes at pericarp maturation (De Castro et al., 2006).

Table 8.6 results suggest that extreme temperatures and rainfall favoured trigonelline production at all bean development stages. Unlike caffeine, trigonelline is synthesized in all parts of coffee seedlings particularly young tissues like the fruit pericarp (Priolli et al., 2008) and declines in ripe fruits after accumulating at the endosperm of coffee seeds. With exception of rapid cell division, high maximum temperatures, many rainy days and mean total rainfall regulated fat production in all bean development stages. Transition from pre-storage to maturation (storage) phase is accompanied by deposition of compounds such as fat.

Beans with high caffeine and chlorogenic acid content had a long roast period and were from vigorous trees as indicated by Table 8.4, reflecting incomplete bean development. Caffeine, a byproduct of chlorogenic acid and purine bases could be produced in response to stress, serve as an antiherbivory and allelopathic compound (Uefuji et al., 2003). Stressful conditions associated with physiological age (Table 5.8) and excessive yield in vigorous trees may produce beans with high

caffeine and chlorogenic acid that roast longer. Caffeine in the fruit pericarp is biosynthesized mainly from adenine intermediate by demethylation and oxidation aided by N-methytransferase group, catalyse and –methyl-(9) nucleoside. Physiological tree age stress may also generate lignified or woody beans that roast longer. Reducing coffee fruit load through longer internodes, fewer fruiting nodes and lower flower induction may improve bean quality. The fact that dense beans included screen size 18, varying bean sizes from young, old and trees with many stems (Table 8.7) suggest that inherent, management and other environmental factors moderated bean weight.

Meanwhile the degradation process could have increased chlorogenic acid and caffeine during roasting (Table 8.4). Phenolic acids, especially depsides, representing various caffeoyl-, feruloyl-, and coumaroylqinic acids, in particular chlorogenic acids or 5-caffeoylquinic acid are contained in coffee beans (Berthaud et al., 1988). Upon roasting, chlorogenic acid and nucleic acid degrade to products such as methylxanthines and purines that synthesize caffeine with a Xanthosine precursor. Other compounds may also degrade to form chlorogenic acid. At dark roasts, 80% of the chlorogenic acid may be lost resulting in a residual CGA content of 2.2-2.4%. (Clifford et al 1985; De Maria et al., 1996) The low bean bromine and high bean zinc contents that increased caffeine content as indicated in Table 8.5 might have resulted from low theobromine production when chlorogenic acid degraded to substances such as libertine, methylliberine, caffeine and theobromine (Poisson, 1988).

In Table 8.7, green bean physical character variability of 8.0-29.25% was explained by morphological traits and biochemical compounds. Bean physical variability of 24.3-50.9% was explained by nutrient elements (Table 8.8) while 5.1-25.3% bean physical variability was explained by rainfall and temperature at bean development stages (Table 8.9). The Results, reported here, therefore suggest that elements soil (lead), leaf (bromine, manganese, potassium) and bean

(potassium, copper, zinc, lead, zinc) explained the most bean physical variability followed by morphological and biochemical compounds and least by rainfall and temperature development stages. Essential elements are core in vegetative and reproductive tree growth. Whereas leaf bromine explained 50.9% of roast bean weight loss variability (Table 8.8) while 48.7% variability for roasted bean volume increase was explained by high bean potassium and bean zinc contents, low leaf manganese, bean copper and bean lead contents. It therefore implies that nutrient elements explain about 50% variability for roasted bean weight and volume. Additionally, screen size 15 bean variability was explained by 24.3% soil lead, leaf potassium and bean zinc contents. Soil texture also influenced bean qualities in that clay loam soil produced significantly less dense beans with a high roast volume increase (Table 4.13).

The different bean sizes portrayed different quality traits derived from trees with different phenotypic traits. For instance large screen size 18 beans had more dry matter, low caffeine and trigonelline derived from productive trees with a few stems, long and narrow leaves (Table 8.7). Beans of screen size 15 and above had high amounts of sucrose and low concentrations of chlorogenic acid and trigonelline, dominantly from trees with many fruits, long and narrow leaves. Other than the good aftertaste, flavour and acidity (Table 8.1), screen size 15 beans had more sucrose and lower quantities of dry matter, caffeine and trigonelline. Trees that produced screen size 15 beans were either young or had many stems with long internodes and many fruits. Screen size 12 and below beans were less dense, had more fat and high amounts of chlorogenic acid and trigonelline (Table 8.4; Table 8.7). Small bean bearing trees had a few fruits, short and broad leaves.

Bean size has been associated with the extent of perisperm development, which is pronounced in shade than sun grown coffee (De Castro et al., 2006). The high hexose to sucrose ratio experienced

over a longer period in shade than sun grown coffee could encourage cell division and elongation in young perisperm and endosperm tissues resulting in larger beans (Muschler, 2001; Vaast et al., 2006). The 75% trees that had broad and open tree canopy as in Figure 4.1a exposed many fruiting nodes to sunlight that caused excessive flowering and fruiting beyond what a tree can adequately sustain to attain potential maximum size, unless under good crop husbandry. As reported earlier (Muschler, 2001; Vaast et al., 2006), increase in height increased bean density possibly because of prolonged bean filling, although at 1400-1500 m above sea level and above, bean size reduced (Table 4.14) with a weak aftertaste (Table 6.7). Long and narrow leaves that were associated with screen size 15 and 18 had possibly better photosynthesis, reduced energy loss through transpiration consequently producing dense beans with good aftertaste, mouth feel, acidity and balance as Table 8.7 shows. In addition, significantly low amounts of soil lead, leaf potassium and bean zinc favoured screen size 15 formation as exhibited in Table 8.8.

Direct association of coffee bean seed density and dry matter indicated in Table 8.7 was also reported on Arabica coffee (Tessema et al., 2011; Vaast et al., 2006). The low caffeine, chlorogenic acid and trigonelline in dense and large beans (Table 8.4; Table 8.7) poses a difficulty in improving antagonistic traits concurrently, particularly trigonelline for its desirable flavour attributes. Lower quantities of caffeine and chlorogenic acid that are bitter in dense beans are likely to offer desirable cup quality. Similarly on Arabica coffee, Tessema et al., (2011) discussed the difficulty in improving negatively associated caffeine and average bean weight traits. Desirable traits with positive effect offer chance of simultaneous improvement.

Except for the development of dense beans that required high minimum temperatures during endosperm stage, roast time, bean roast volume, screen sizes 18, 15 and 15 and above were influenced by rainfall (mean, total, rainy days) at different stages of bean development (Table 8.9).

Development of dense beans at minimum temperatures (Table 8.9) concurs with reports that indicated low temperatures allowed for longer and better bean filling (Vaast et al., 2006). The results of this work, implicate rainfall as a major determining factor for bean size and roasting qualities. A well distributed rainfall of 120 to 2000 mm at about 24°C to 30°C and humidity of 80-90 % over a period of 9 months ideally sustains newly set fruits of Robusta coffee (Wrigley, 1988). Bean size is also associated with the extent the bean perisperm tissue develops to occupy the entire volume of the locule which is more pronounced under shaded plants (De Castro et al., 2006). Seed physiology and development reportedly varied according to the position of fruits within plagiotropic branches and the light they received that influenced the biochemistry and gene expression (De Castro et al., 2006; Vaast et al., 2006)

Tree morphological parameters were explained by 13.9-44.4% essential elements namely soil tin, soil zinc, soil copper, soil calcium, soil iron, leaf copper, leaf manganese, bean copper and bean manganese (Table 8.10). Rainfall and temperature at different bean development stages explained 3.12-18% of tree morphological character variability (Table 8.11). In Table 8.10, soil copper, soil zinc, soil iron, bean manganese and leaf copper, influenced plant growth as low amounts of soil zinc and high soil copper increased tree girth while high soil zinc and bean copper increased tree vigour. In Figures 4.5; 4.6a, tree vigour was found positively correlated to productivity and many fruits and negatively with stems. As essential element contribution to tree development, high contents of soil and bean copper increased stem numbers, low soil and leaf copper generated long and broad leaves while high soil copper, low soil iron and bean manganese produced long internodes. To highlight the importance of soil, leaf and green bean interactions (Table 7.2; 7.3; Figure 7.1; 7.2; 7.3), regression of soil elements (potassium, calcium, tin, manganese, iron, copper, zinc, lead and bromine) on similar bean elements showed significant effects of soil tin, calcium, bromine and

manganese on bean elements (Table 7.2) and lead, potassium, manganese, tin, bromine and copper on leaf elements (Table 7.3). Element interactions affect availability and concentration in soil, leaf and green bean. Micronutrients such as zinc, copper, iron and manganese are required by the coffee plant in small amounts for normal functioning of plants (Iloyanomon et al., 2011). Other than environment effects on phenotypic traits, Leroy et al., 2008 found QTLs in one of the years ranged from 34%-57% for yield, 25%-35% for bean size, 22%-35% for chlorogenic acid content, 29%-81% for sucrose and trigonelline content and 30% -55% for liquor acidity and bitterness.

Many rainy days at pin head stage enhanced tree production capacity most likely by sustaining young beans (Table 8.11). Robusta coffee requires few rainy days with minimal backing showers of 2.5 to 4.0, lest newly developed fruits dry up or get infected in high humidity thus affecting the final yield and tree productivity. The high tree vigour at pinhead with many rainy days and full bean formation with low mean and high total rainfall possibly reflected existing small and fully developed beans numbers. Rainfall requirements depend on the retention properties of the soil, atmospheric humidity, cloud cover as well as cultivation practices. The many stems induced with a few rainy days during endosperm stage (Table 8.11) may have resulted from assimilate supply, although physical and pest leaf damage affects shoot growth. Removing excess stems limits nutrient competition with the developing coffee bean at full formation stage. The results from this work indicated that long leaves grew at high minimum temperatures during bean full formation, with many rainy days and low minimum temperature at rapid cell division (Table 8.11), implying leaf growth may require low temperatures. But low temperature at pin head stage and many rainy days at rapid cell division were essential for broad leaf development.

CHAPTER NINE: GENERAL CONCLUSIONS AND RECOMMENDATIONS

9.1 CONCLUSIONS

The genetic and phenotypic variability of *C. canephora* in traditional growing areas and effect of important environment factors on quality traits have extensively been investigated in this study. The SSRs makers used in this study were polymorphic, efficient in detecting variability to the extent of rare alleles and differentiation. The three genetic diversity groups of Ugandan cultivated *Coffea canephora* that had no relationship with the locations they originated from and were quite different from those from Western and Central Africa. The within individuals variability of 54.05% implied cultivated genotypes were diverse. Germplasm collection accessions had limited variability. Among the parameters of genetic diversity measured, there was no negative association indicating that there was no antagonism. Cultivated Robusta coffee was dominantly out crossing, was moderately crossing among genotypes from different locations and evolving. Kamwenge had the highest total number of different alleles, information index and rare alleles.

Most cultivated Uganda *C. canephora* comprised 88% “nganda” and “erecta” landraces and 12% research improved materials of which 34% of the trees were above 40 years. Though controlled crosses and hybrid types were significantly different for morphological traits, were restricted to mostly single different genetic diversity groups, reflecting limited variations within each type and ineffective to contain disasters like disease epidemics. ‘Nganda’ and ‘erecta’ Robusta coffee types were not genetically distinct but significantly different for morphological traits. About 61% of Robusta coffee beans were average screen size 15 while 19% were larger.

Variability in green bean biochemical compounds was genetically and environmentally influenced. Genotypes in the specific 3 genetic diversity groups did not constitute biochemical compound groups of their own possibly due to environmental influence on the latter. The significantly

different dry matter and caffeine content among the four multivariate diversity groups implied dense beans with low caffeine can be selected. Ugandan Robusta coffee had more fat (10.44 to 15.94% of dry matter), chlorogenic acid (10.88 to 15.64 % of dry matter) that potentially provides more bean flavor. Dry matter, sucrose, fat, caffeine, chlorogenic acid and trigoneline contents of Ugandan cultivated Robusta coffee was quite different from that in the CIRAD data base reflecting high species variability for the compounds and pointing out that CIRAD had not earlier evaluated Ugandan Robusta coffee. The near infra red method of fingerprinting coffee bean biochemical compounds is efficient and economically viable.

About 84% of Ugandan Robusta coffee organoleptic cup was of premium grade. The four organoleptic cup diversity groups were significantly different for fragrance, aroma and flavour, reflecting ease to select for the traits. Regression coefficients of cup attributes and overall cup assessment rated balance (0.90) as the most important. Cupping protocol was able to differentiate biochemical compounds in brewed coffee.

Mean optimum expression of Robusta coffee phenotypic quality traits was 1200 m.a.s.l and 30 to 35 years. Robusta coffee types, soil texture, locations, tree age and altitude range had a significant contribution to the performance and constitution of phenotypic traits and nutrient elements at varying magnitudes. Cultivated *C. canephora* had immense genetic and phenotypic variability that can be used to select representative traits for conservation and improvement of Robusta coffee desirable agronomic traits, including quality in consideration with the environment.

The soils where Robusta coffee is grown in Uganda were significantly different for iron content and deficient in potassium, calcium, manganese, iron, zinc, copper, lead, bromine and tin elements. Potassium was most in beans and likely to support activities that improve deposition of biochemical

compounds that improve cup quality such as carbohydrates, fat, proteins. High concentration of calcium in leaves is important in sustaining leaf cell activities that include photosynthesis. EDXRF is a rapid, accurate, nondestructive and easy to use powerful tool recommendable for research for direct determination of micro and macro nutrients elements across the periodic table in solid, liquid, powder and thin film form.

Though I did not analyze genotype x environment interactions, Robusta coffee traits were influenced by genotype and environment. Quality traits were singly or in combination influenced by phenotypic traits, elements, rainfall and temperature at bean development stages. Soil, leaf and green bean elements explained the highest variability in organoleptic cup (6.3%-22.1%), bean physical characters (24.3-50.9%) and tree morphology (13.9-44.4%). Most biochemical variability was explained by 3.7 to 35.5% rainfall and temperature bean development stages. Average screen size 15 beans had better aftertaste, acidity, flavour, sucrose and less dry matter, caffeine and trigonelline contents and may be most desirable.

Overall, both genetic and phenotypic methods were able to characterize and determine the structure of grown *C. canephora* at different magnitudes. PCA clustering algorithms and UPGMA hierarchical distance method easily clustered DNA data. PCA analysis successfully or unsuccessfully clustered phenotypivariables necessitating nonhierarchical K means analysis of variance and factorial step discriminant analysis to unearth hidden relevant information. Genetic diversity using phenotypic traits does not reveal or interpret neither alleles nor ancestral relationships but a reflection of environment and plant inherent constitution. Even if traditional breeding is envisaged expensive, time consuming and very dependent on environment, morphological characters are important when choosing parental materials for breeding and exploitation of useful traits even in molecular studies. Coffee breeding strategies should consider complementing conventional

methods that consider influence of environment on inherent traits with molecular methods that investigate with non-genetic variance to improve efficiency and speed.

9.2 RECOMMENDATIONS

Given that 80% of coffee production in Uganda constitutes *C. canephora* with a total economic benefit of about 60%, it is imperative thatUganda plans an *in situ* and *ex situ* conservation strategy to rescue the high existing variability from looming erosion and enrich genetically limited germplasm collections. A diverse Robusta coffee gene pool not only saves time wastage and research cost of sourcing variability elsewhere but increases chance of research success. Borrow valuable *ex situ* and *in situ* conservation ideas from Cote D' Ivoire where wild and cultivated classified genotypic groups of Robusta coffees were conserved as reference living collections of wild forms, local varieties, populations from farms and selected materials. Madagascar has also conserved another 25 diploid coffee species *in situ*. Conservation accessions may include those with high allele frequencies for breeding purposes and rare alleles for their uniqueness. Forest collections, farmer landraces, research selections, research hybrids, introductions and collections from other regions like the North, North West that were not represented in the study need not be neglected. Ecological niches with diverse genotypes, particularly in Western highlands of Kamwenge, Kyenjojo, Bundibudyo, Kabarole and Hoima in Lake Albert Crescent region should be considered for both *in situ* and *ex situ* conservation.

To derive in-depth understanding of the country's Robusta coffee genetic resources, a country wide evaluation of forests such as Zoka forest and farmer landraces from areas such as West Nile, Acholi and Karamoja region that had not been evaluated should be planned. The three genetic diversity groups from DNA analyses should be designed for further studies on association between characters or multi-locus structure of groups. Enzymes that are associated with specific genes in the synthesis

of different biochemical compounds that influence coffee quality and human health should be designed. Association studies should identify complex traits of agronomic importance.

Crucial to strategic *in situ* and *ex situ* conservation is a study to investigate the potential value of conserving *C. canephora* genetic resources. To best exploit and conserve available genetic diversity, collaborative work with regional and international organizations may be formulated. Genotypes with superior phenotypic traits either in morphology, green bean physical characters, biochemical compounds or organoleptic cup traits should be evaluated in replicated multi locational trials with uniform but diverse treatment applications to refine selection criteria. Desirable agronomic traits such as growth parameters, moisture stress, disease and pest tolerance, bean quality, yield and conversion ratio of ripe cherry to clean green beans ought to be assessed. Specific and general combining ability of agronomic traits could increase selection efficiency. Beans with defects attributed to inherent traits, physiology or environment such as hollow, wrinkled and pea berries should be studied. Effect of poor crop husbandry, soil amendments, processing practices among others on coffee quality should be evaluated.

To generate variability and select for desirable traits in a genotype, a hybridization program should utilize result genetic and phenotypic diversity groups to maximize segregation. Hybridization products should be used to map and identify trait specific genes. Highly heritable traits like bean physical shape, average weight, fat and caffeine content should be recommended as the first step in selection and quality improvement. Due to coffee biannual pattern particularly in traditional varieties, smaller multi locational experiments over at least two consecutive years should be designed to determine the effect of heavy and light crop on green bean physical characteristics, green bean biochemical compounds and organoleptic cup qualities. Economic and environmental effects of coffee tree shade sought to be conducted.

Since Ugandan Robusta coffee was found to be of premium to specialty grade with some genotypes attaining specialty grades, the coffee sold should also attain average prices and above. Armed with the knowledge on organoleptic cup profiles and soil status, farmers should improve crop husbandry, harvest and post harvest processing to enable premium cup grade attain fine grade Research institutions could guide farmers to maximize use of quality profiles and genotypes with superior quality traits to improve price offers. As a means of value addition, farmers should harvest and process landraces separately from improved Robusta coffee and market independently. Support for grading physical bean and organoleptic cup characteristics should be considered up to farm level. Farmers are also urged to plant shade trees to improve coffee bean qualities and ensure annual yields. Farmer field schools and collective marketing should be encouraged and supported to increase productivity, quality and income. To minimize loss of farm genetic diversity through aging trees, farmers ought to be educated and advised on importance of genetic resources and the need to replace old trees with clones of same trees. The practice of collecting planting materials from the wild should be encouraged to sustain or enhance gene pool.

All analyses techniques used here revealed a wide range of genetic and phenotypic variability implicating their usefulness in diversity studies and their use is further recommended. Unlike molecular marker variability results that lacked environmental influence, the rest of the investigation tools revealed phenotypic diversity that is influenced by genetic and environmental factors. For in depth understanding of variability in Robusta coffee quality, analytical tools that investigate variations in soil structure and element content, vegetative characters to bean traits as well as prevailing environment are inevitable and are complementary. Though costly to acquire NIR and EDXRF machine, the quality and quantity of information obtained from them outweighs their costs.

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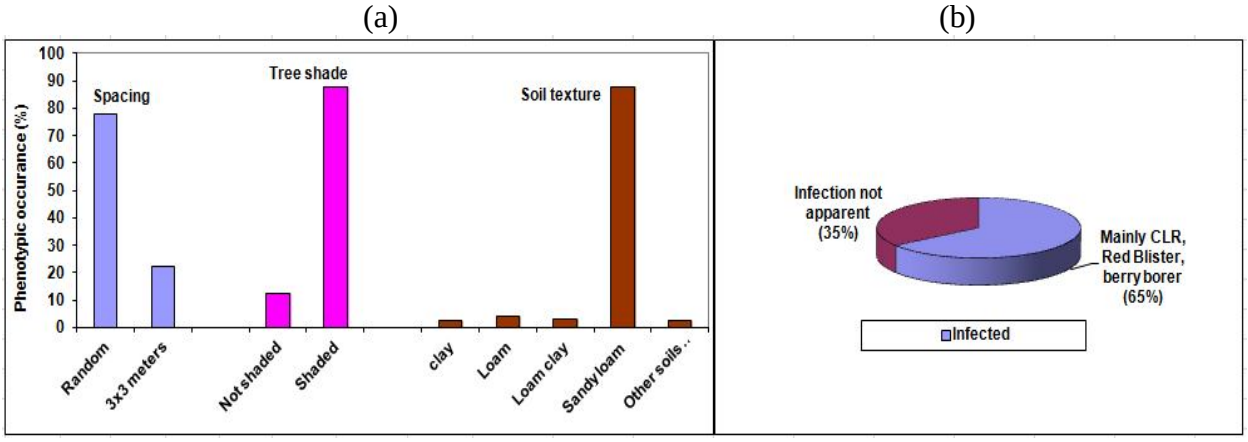
APPENDICES

Appendix 1: Allele frequencies distribution in Robusta coffee accessions

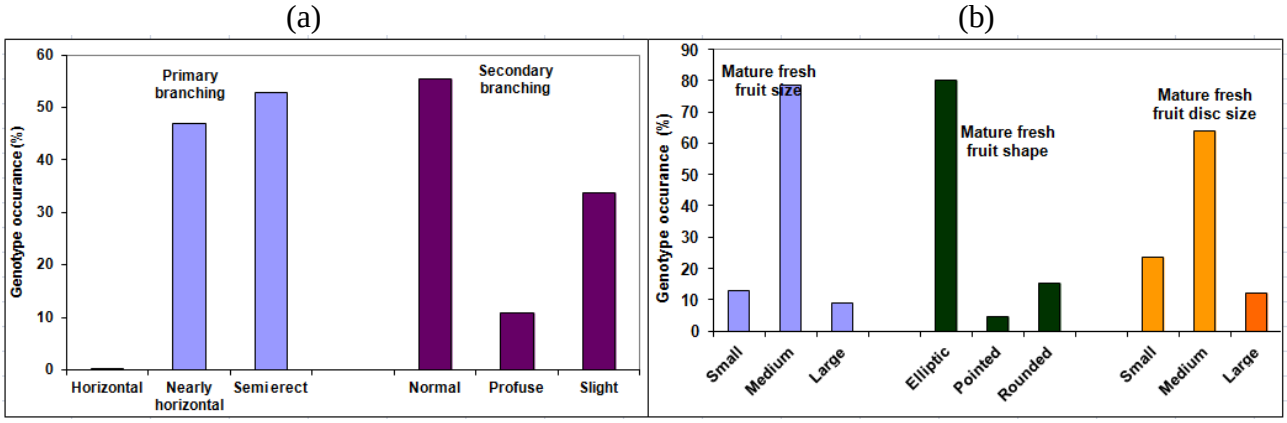
Markers	≤ 0.10	0.1 ≤ 0.20	0.2 ≤ 0.30	0.30 ≤ 0.40	0.40 ≤ 0.50	0.50 ≤ 0.60
DL026	29	62	119	0	0	266
355	134	332	0	0	0	0
358	44	236	0	0	210	0
364	89	0	63	0	130	0
368	272	48	0	144	0	0
384	0	81	119	0	0	245
394	113	147	0	0	0	264
429	127	90	138	163	0	0
442	202	0	103	185	0	0
445	93	0	150	0	0	279
456	522	0	0	0	0	0
461	130	243	147	0	0	0
471	93	295	130	0	0	0
477	101	73	0	354	0	0
501	125	173	0	0	218	0
753	102	140	266	0	0	0
837	152	138	0	0	218	0
790	130	382	0	0	0	0

Appendix 2: Cultivated Robusta coffee acreage at varying levels of missing trees

without gaps	33% missing trees	50% missing trees	Farms ≥3.5 acres excluded without gaps
0.05	0.03	0.025	0.05
13	8.71	6.5	2.5
1.14	0.76	0.57	0.90
1.50	1.01	0.75	0.63
166.0	111.22	83.0	125.5
146	146	146	140



Appendix 3a, b: Cultivated Robusta coffee (a) tree spacing, shade prevalence and soil texture (b) plant health status.



Appendix 4a, b: Cultivated Robusta coffee (a) branch inclination and numbers (b) bean size, shape and fruit disc size

Appendix 5: Cultivated Robusta coffee type, tree, primary and secondary branch characteristics.

	Tree shape			Tree growth habit			Tree height			Angle of primaries			Secondary branching			Primary strength		
	con	interm	cylind	open	normal	compact	tall	med	short	erect	near horiz	horiz	prof	normal	slight	thin droop	Av.	thick strong
Com	1	38	5	17	27	0	34	10	0	23	20	1	4	30	10	1	29	14
Erecta	4	155	33	73	117	2	149	43	0	102	90	0	11	107	74	1	71	120
Hybrid	0	15	1	2	14	0	6	9	1	8	8	0	0	15	1	0	16	0
Nganda	6	151	67	142	79	3	178	40	6	119	105	0	37	112	75	19	120	85
Total	11	359	106	234	237	5	367	102	7	252	223	1	52	264	160	21	236	219
Sum	476			476			476			476			476			476		

Appendix 6: Grown Robusta coffee type, leaf and ripe fruit characteristics

	Leaf flush colour			Adult leaf colour			Leaf shape		Leaf surface			Fruit size			Fruit shape			Fruit disc size		
	bronze	light bronze	green	dark green	green	light green	ovate	round	undul	interm	flat	large	med	small	point	ellipt	round	large	med	small
Com	1	28	15	1	38	5	44	0	0	22	22	17	26	1	0	40	4	3	37	4
Erecta	34	118	39	16	146	30	188	4	5	132	55	14	151	27	8	154	30	31	120	41
Hybrid	3	12	1	5	7	4	16	0	8	8	0	0	16	0	1	8	7	6	8	2
Nganda	41	141	42	25	178	21	219	5	7	168	49	11	180	33	13	180	31	18	140	66
Total	79	299	97	47	369	60	467	9	20	330	126	42	373	61	22	382	72	58	305	113
Sum	475			476			476		476			476			476			476		

Key to Table 4: con=conical; interm=intermediate; cylind=cylindrical; med=medium; horiz=horizontal; prof=profuse; Av.=average; undul=undulating; med=medium; ellip=elliptic

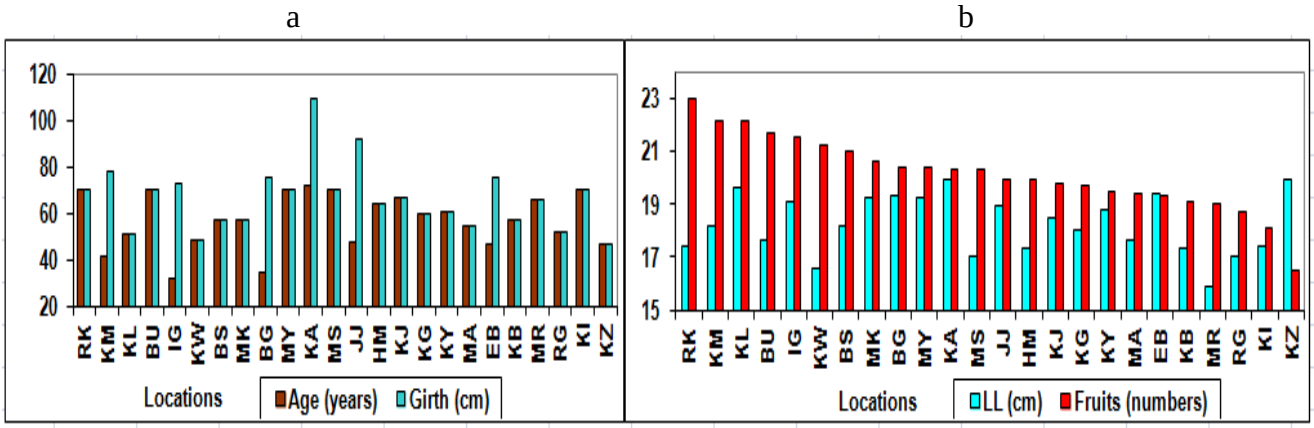
Appendix 7: ANOVA of grown Robusta coffee types morphological characters

				Significant Robusta coffee types			
Mxtrs	R^2	v.r	p v	Coffee type	coefficient	s.e	p v
Age	0.09	25.9	****	com, hybrids	-25.7, -25.4	5.28, 8.29	****, ****
Girth	0.07	11.8	****	com, hybrids	-27.8, -27.3	0.10, 0.16	****, ***
PC	0.04	6.6	****	com	0.46	0.11, 0.17	****, *
Vigour	0.04	5.9	****	com, hybrids	0.37, 0.04	0.10, 0.16	****, **
Stems	0.07	11.9	****	com, erecta	-2.54, -1.3	0.57, 0.34	****, ****
				Hybrids	-3.38	0.90	****
IL	0.04	6.5	****	com, erect	0.41, 0.22	0.19, 0.11	**, **
				hybrids	-0.89	0.29	***
LL	0.04	6.2	****	com, erecta	1.27, 0.44	0.41, 0.24	***, *
				Hybrids	2.05	0.64	***
Fruits	0.03	4.8	***	hybrids	-3.43	1.18	***
F Stat; F3, 472							

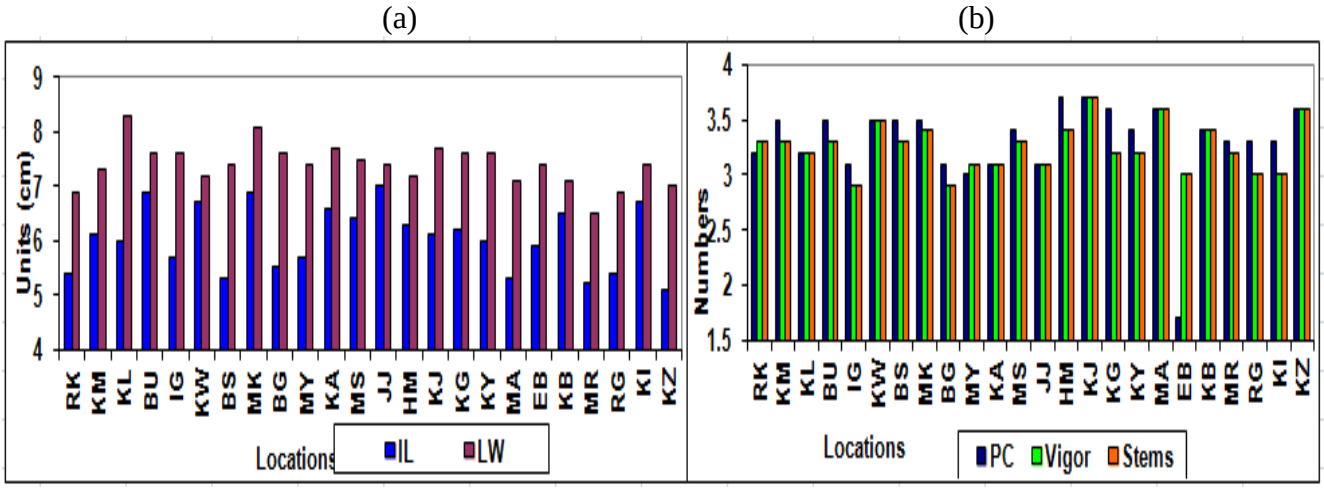
*, **, ***, **** significant at 0.09, 0.05, 0.006, 0.0006 levels of probability

Appendix 8: Relationships of cultivated Robusta coffee phenotypic characters

	Age	Girth	PC	Vigour	Stems	IL	LL	LW	Fruits	Alt
Age	0									
Girth	0.76***	0								
PC	-0.24***	-0.11*	0							
Vigour	-0.15**	-0.10*	0.63***	0						
Stems	0.18***	0.26***	-0.08	-0.12*	0					
IL	0.07	0.03	-0.03	-0.02	0.00	0				
LL	0.06	0.05	-0.05	0.12*	-0.27***	0.21***	0			
LW	0.00	0.00	-0.05	0.07	-0.14**	0.30***	0.63***	0		
Fruits	0.00	-0.01	0.25***	0.27***	-0.16**	0.11***	0.12***	0.18***	0	
ALT	-0.09**	-0.09*	0.11	0.14**	-0.05*	-0.12**	-0.05	-0.04	0.00	0



Appendix 9a, b: Location comparison of cultivated Robusta coffee (a) mean tree age, girth (b) leaf length and fruits



Appendix 10a, b: Comparison of farmer Robusta coffee mean tree (a) inter node length, leaf width (b) production capacity, vigour and stems at locations

Appendix 11: Bean defects found in farmer Robusta coffee green bean study samples.
Key to Figure 5.2: plant debris (1), pale beans (2), deformed beans (3), hollow beans (4), stone (5), beans rotten in dry cherry (6), pest and disease infested (7), pea berries (8), hulls and beans stuck to hulls (9) and chipped beans(10).

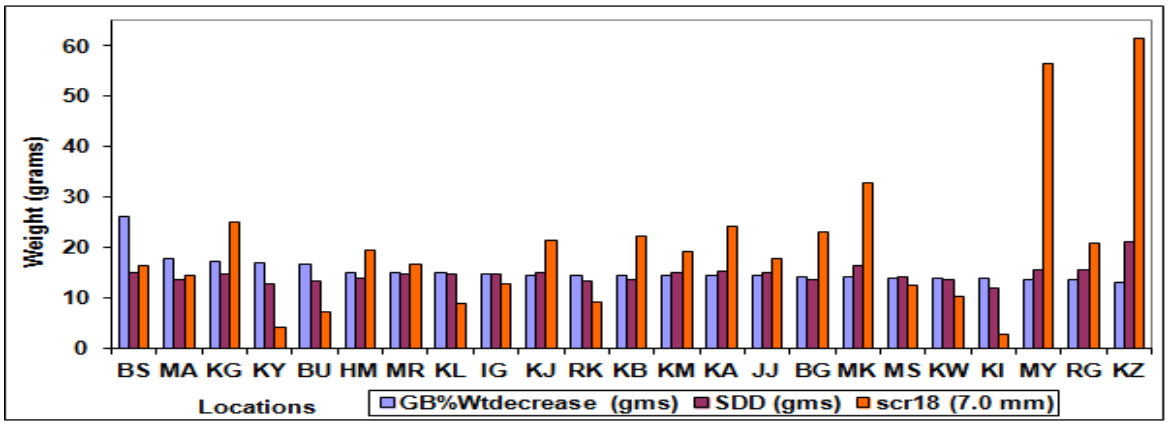
Appendix 12: Correlation coefficients for 204 farmer Robusta coffee green bean physical characters

	Alt	Years	RTPG	GB	RB	SDD	scr18	Scr≥15	scr15	S≤12
Altitude	1									
Years	-0.08	1								
RTPG	-0.08	0.18	1							
GB	-0.09	-0.15	-0.04	1						
RB	0.01	0.00	0.39**	-0.31	1					
SDD	0.03	-0.08	-0.14*	0.03	-0.12	1				
scr18	0.06	-0.05	-0.08	0.03	-0.12	0.77***	1			
Scr≥15	-0.03	-0.09	-0.37**	0.08	-0.20	0.65***	0.53***	1		
scr15	-0.09*	-0.04	-0.30**	0.04	-0.08	-0.12	-0.48***	0.49***	1	
S≤12	0.03	0.09	0.37**	-0.08	0.20	-0.65***	-0.53***	-1.00	-0.49***	1

Appendix 13: Effect of altitude and tree age on farmer Robusta coffee green bean physical characters

Regression statistic summary									
Bxtrs	predictors	coefficient	s.e	predict	d.f	v.r	F pr	t pr	R^2 (%)
RTPG	Alt+age	3E-4	1E-4	age	2,201	3.81	**	**	2.7
GB	Alt+age	-0.04	0.02	age	2,201	3.56	**	**	2.5

Key: Bxtrs=bean characters; predict= significantly different predictors; r.c=regression coefficient; s.e=standard error; d.f=degrees of freedom; v.r=variance ratio; F.p=Fisher probability test; t.p=student t test; var=% variance, RTPG=roast time per gram; GB=roasted green bean % weight decrease.



Appendix 14: Comparison of farmer Robusta coffee mean green bean roast weight decrease, seed density and screen size 18 at different locations.

Appendix 15: Green bean physical character diversity groups accessions derived from K means analysis

Group 1				Group 2		Group 3
BG05	JJ08	KL12	MK04	BG17	KW06	BU08
BG11	JJ09	KL15	MK05	BS04	KW07	HM18
BG12	JJ13	KL18	MK07F	BS11	KW17	IG14
BG14	JJ15E	KL20	MK10	BU01	KW19	KA30
BG16	KA01G	KM02B	MK12E	BU10	KY05	KA36
BS01	KA16	KM04C	MK13	HM13	KY11a	KG15E
BS06	KA22	KM05	MS07	HM17	KY13	KG21
BS08	KA29	KM08	MS08D	HM22	KY17C	KM03A
BS15	KA31	KM12	MS10	HM26	KZ18	KZ07
BS16	KA39	KM14	MS11	JJ07	MA03	KZ09
BS18	KA43	KM17	MY01	KA03	MA05	KZ11
BU04	KA47	KM19	MY16	KA12	MA15	KZ12
BU05	KB03D	KM21	RG01	KA37	MA17	KZ15
BU06	KB04G	KW02	RG02	KA42	MR10	KZ16
BU12	KB09	KW04	RG03	KA49	MR11	MA10
BU13	KB11	KW08	RG06	KA52	MR12	MK08
BU14	KB18	KW11E	RG07	KB02	MR13	MK11
BU15	KG01	KW15	RG15	KB17	MR19	MR01D
HM04C	KG03	KW22	RG17	KG02	RK09G	MR04
HM06	KG05	KY03	RK01	KG09	RK15	MY08
HM07	KG13D	KY12	RK03	KG18	RK16	RG08
HM09	KI02	KY16	RK05	KI05	RK17	RG10
HM10D	KI16	KY20	RK06G	KI10	RK18	RK02

HM12	KJ05	KZ14	RK10	KI19		
HM16	KJ10	MA02	RK13	KJ01a		
IG04	KJ16G	MA04	RK14	KJ02		
IG07	KJ17D	MA08		KJ23		
IG13	KJ21	MA11		KL04		
JJ01	KJ26	MA12G		KM16		
JJ02	KL01	MA16		KM22		
JJ03	KL03	MA18		KM23		
JJ05	KL08	MK01		KW01		
JJ06	KL10	MK03		KW03		

Appendix 16: Farmer Robusta coffee accessions that had high green bean physical character variability rating with the principal component analysis (squared cosines of the observations)

F1						F2				F3		F4	
Screen size ≥15, screen size ≤12, seed density						Screen size 18				GB%WTdecrease RB%VLincrease		RTPG	
KL04	0.97	RK17	0.70	KW22	0.54	MS07	0.94	KG15E	0.59	KG13D	0.69	BS08	0.62
MR10	0.93	KA47	0.70	MR04	0.53	JJ08	0.89	KZ09	0.59	RG17	0.68	BS15	0.46
RK16	0.93	MA12G	0.69	KJ16G	0.53	HM04C	0.87	JJ03	0.58	BU15	0.65	JJ05	0.42
KZ14	0.91	IG14	0.69	BU01	0.53	KY16	0.87	MA05	0.58	MA04	0.65	KY12	0.40
RK18	0.91	HM09	0.69	BG05	0.52	KL15	0.85	KJ21	0.58	BS18	0.62	KA42	0.39
MR13	0.90	KM21	0.68	KA30	0.51	MK04	0.84	JJ13	0.57	KY11a	0.56	KW07	0.38
KM23	0.87	HM12	0.67	KZ07	0.51	BS11	0.84	MR01D	0.57	HM16	0.55	RG10	0.38
KY13	0.87	HM26	0.67	MR19	0.49	KL12	0.83	IG04	0.57	KI02	0.53	KM05	0.35
KI19	0.87	JJ02	0.66	KM02B	0.49	KG18	0.83	MK08	0.56	JJ01	0.50	KG21	0.34
KM04C	0.86	BU10	0.66	KJ17D	0.49	KG03	0.81	KL03	0.53	KM19	0.49		
RK15	0.86	BG17	0.64	MK12E	0.48	RK14	0.79	KY20	0.52	KM05	0.48		
KJ01a	0.86	KG09	0.64	KZ18	0.48	KY03	0.76	KM03A	0.51	RG02	0.47		
HM22	0.85	MK05	0.64	KY17C	0.47	IG07	0.73	KY17C	0.50	MA11	0.47		
KY05	0.83	MR12	0.62	KW17	0.47	HM18	0.73	BU04	0.49	KB09	0.47		
RG15	0.82	KA36	0.62	BG12	0.47	MY08	0.73	KZ12	0.49	KG05	0.46		
HM13	0.82	BG14	0.62	KA37	0.46	KB11	0.72	KZ16	0.49	RK13	0.45		
RK09G	0.80	BU14	0.61	KZ15	0.45	BU12	0.72	HM07	0.48	BS01	0.45		
BG11	0.80	KA39	0.60	KM12	0.44	MK03	0.71	KZ11	0.48	JJ05	0.45		
RG08	0.79	KM16	0.60	BS06	0.44	RK03	0.68	MA18	0.47	KG01	0.42		
KW06	0.78	KM14	0.60	HM06	0.44	MA02	0.68	KA12	0.45	BS04	0.41		
KW02	0.77	KW08	0.59	KA52	0.43	RG06	0.68	BS16	0.45	BS15	0.40		
HM17	0.77	KJ10	0.59	KZ16	0.43	KL01	0.66	KA03	0.44	KY20	0.40		
MK07F	0.77	IG13	0.59	KJ23	0.42	MA08	0.66	KL10	0.44	MS10	0.38		
KW11E	0.75	MK13	0.59	KW01	0.42	BU05	0.65	RG01	0.44	MK01	0.37		

MY01	0.75	KB17	0.59	KY11a	0.42	KW19	0.64	KJ17D	0.43	KA43	0.36		
KI10	0.74	MK01	0.59	KZ11	0.40	MK11	0.64	KW04	0.43	IG04	0.35		
MA17	0.74	KA22	0.58	MK10	0.40	RK02	0.64	RK10	0.43	BS16	0.34		
KA16	0.73	KL18	0.58	KB18	0.40	KB04G	0.64	KB02	0.42	KB03D	0.34		
KA29	0.73	MA15	0.56	MS08D	0.40	KL20	0.63	JJ07	0.42	KW01	0.34		
BU13	0.73	KI05	0.56	KA31	0.40	JJ09	0.62	KG01	0.42	KL08	0.32		
KW03	0.73	HM10D	0.55	JJ15E	0.39	RK06G	0.61	KJ16G	0.41	RG03	0.32		
KG02	0.72	BU08	0.55	MA16	0.39	KA01G	0.61	RG07	0.40	KW22	0.32		
KM22	0.71	KJ02	0.54	KL10	0.38	KW15	0.60	MS10	0.39	BS06	0.31		

Appendix 17: Green bean biochemical compound diversity groups accessions derived from K means analysis

Group 1				Group 2			Group 3				Group 4		
BG01	KA59	KL11	MR19	BG03	JJ13	KJ17D	BG08	JJ08	KW25	RG05	BG14	KB01E	KY08
BG02	KB13	KL12	MS01	BG04	KA03	KJ18A	BG14	KA02	KY07	RG06	BS03	KB04G	KY18
BG06	KB13	KL18	MS03	BG05	KA07	KJ25	BG15	KA17	KY14E	RG09	BS17	KB05	KZ07
BG09	KB02	KM07	MS07	BG07	KA08	KJ26	BG16	KA28	KY01F	RG10	BS01	KB08	KZ09
BG10	KB06	KM08	MS10	BG17	KA11	KL06E	BS02	KA29	KZ06	RG14	BS11	KB09	KZ10
BG11	KB07	KM11	MS12	BU01	KA13	KL13	BS07	KA56	MA04	RG17	BS15	KB18	KZ11
BG12	KB17	KM14	MS05	EB01	KA21	KL14	BS09D	KB10	MA05	RG18	BU02	KG13D	KZ15
BG13	KG02	KM15	MY03	EB07	KA22	KL16	BS10	KB11	MA06	RG07	BU06	KG15E	KZ16
BS04	KG04	KM17	MY04	EB08	KA25	KL02	BS12	KB12	MA08	RG11	BU12	KI02	KZ17
BS05	KG06	KM18	MY06	EB09	KA30	KM03A	BS13	KB14	MA09	RK01	BU16	KI09D	KZ02
BS08	KG07	KM20	MY07	HM01	KA37	KM04C	BS14	KB15	MA10	RK04	EB03B	KI10	KZ03
BU03	KG08	KM22	MY08	HM02	KA38	KM05	BS15	KB16	MA12G	RK08	EB04D	KI03	MA01
BU04	KG11	KM23	MY09	HM03	KA40	KM09	BS16	KG12	MA15	RK09	EB06	KI11	MA02
BU11	KG18	KM01	MY11	HM14	KA42	KM13	BS18	KG01	MA18	RK13	HM06	KJ14E	MA14
HM04C	KG19	KM19	MY13A	HM23	KA43	KM05	BS19	KG17	MA03	RK14	HM20	KJ20	MA16
HM05E	KG20	KW04	MY14	IG09	KA44	KM12	BS06	KI05	MA07	RK15	HM21	KJ05	MK03
HM08	KG21	KW08	MY15	IG14	KA47	KM16	BU05	KI13	MA11	RK16	HM06	KJ10	MK08
HM13	KG05	KW15	MY16	IG16	KA48	KY02	BU07	KI16	MR05	RK17	HM07	KJ10	MK09
HM19	KI06	KY11	MY17	IG17	KA49	KY03	BU08	KI20	MR06	RK18	HM09	KJ15C	MK15D
HM24	KI12	KY12	RG02	JJ01	KA50	KY05	BU09	KJ01A	MR08		HM12	KL03	MK02
IG03	KI15	KY16	RG08	JJ04	KA52	KY09	BU10	KJ22	MR09		HM18	KL07	MK05
IG04	KI19	KY17C	RG13	JJ05	KA54	KY10	BU13	KJ04	MR10		HM26	KM02B	MK07F
IG06	KI21	KY19	RG16	JJ07	KA01	KZ04	BU14	KL09	MR11		IG05	KM10	MK12E
IG07	KI18	KY20	RK05	JJ09	KA31	KZ08	BU15	KL10	MR18		IG11	KM21	MR01D
IG13	KJ03	MA13	RK11	JJ11	KB03D	KZ12	EB02	KL15	MR04		JJ03	KW11E	MS09
IG15	KJ06	MA17	RK12	JJ12	KG03	KZ13	EB05	KL17	MR07		JJ15E	KW12	MY01
JJ02	KJ12	MK06		JJ13	KG09	KZ14	EB10	KW01	MR13		KA12;53	KW13	RG12
KA05	KJ13	MR02G		JJ14	KG17	KZ18	HM10D	KW03	MS02		KA16	KW14	RG04
KA19	KJ21	MR03		JJ16	KI04	MK01	HM11	KW06	MS06G		KA22	KW17	RG15
KA23	KJ23	MR12		JJ17	KI14	MK04	HM17	KW07	MS08D		KA27	KW20	RK02
KA24	KL01	MR14		JJ16E	KJ02	MK10	HM22	KW10G	MS11		KA39	KW21	RK03
KA36	KL04	MR15		JJ10F	KJ09	MK11	HM16	KW16	MY12		KA46	KY13	RK06G
KA45	KL05	MR16		JJ06	KJ11	MK13	IG08	KW19	RG01		KA55	KY15G	RK09
KA51	KL08	MR17			KJ16G	MK14G		KW22	RG03		KA09	KY06	RK10

Appendix 18: Farmer Robusta coffee accessions that had high green bean biochemical compound variability rating with the principal component analysis (squared cosines of the observations)

F1						F2				F3			
Caffeine, trigonelline						Chlorogenic acid, fat				**Dry matter, sucros**			
MA04	0.98	KW22	0.75	MK01	0.63	KL17	0.95	KJ14E	0.70	KA59	0.88	MR02G	0.60
KG17	0.96	JJ14	0.74	MS11	0.63	JJ03	0.93	MR13	0.69	MS01	0.87	BU16	0.60
HM14	0.96	IG15	0.73	KW06	0.63	MR05	0.92	HM09	0.68	KM21	0.86	IG16	0.59
MK11	0.94	BU05	0.73	KB07	0.62	KY10	0.92	MA10	0.68	KA43	0.85	KZ12	0.59
RK16	0.94	JJ16	0.73	MY13A	0.62	KZ09	0.91	MR12	0.68	MK02	0.85	HM23	0.59
KJ18A	0.94	KA52	0.72	BU03	0.62	KZ07	0.90	MY12	0.68	HM07	0.81	MK12E	0.59
KB14	0.94	BS06	0.72	MA02	0.62	MK13	0.88	MS10	0.68	KY02	0.79	HM16	0.59
IG09	0.91	KG05	0.72	RK15	0.61	KB16	0.88	KW03	0.67	KJ11	0.79	KA29	0.58
KM19	0.90	RG03	0.72	HM02	0.61	MS03	0.88	KG02	0.67	KY17C	0.76	BS17	0.57
BU10	0.88	KG13D	0.72	EB05	0.61	MR08	0.87	KA47	0.66	KJ05	0.76	MS09	0.57
MR01D	0.87	KG06	0.71	MA03	0.59	KA38	0.85	KL09	0.65	KY06	0.75	BG06	0.56
RG04	0.86	KW19	0.71	MY04	0.59	KZ15	0.82	MK08	0.65	KI11	0.74	RK09	0.56
BS02	0.86	BS15	0.71	MS02	0.58	KA21	0.82	KG19	0.65	KY20	0.74	MA05	0.55
JJ10F	0.84	IG04	0.70	EB04D	0.58	KB01E	0.82	MY07	0.64	BG07	0.72	KM22	0.55
KL08	0.83	BG04	0.70	BS18	0.58	KA30	0.82	BS04	0.64	HM08	0.72	KW20	0.53
KG17	0.83	KG20	0.69	HM21	0.57	KZ17	0.81	KA42	0.62	KW14	0.71	MK09	0.53
JJ02	0.82	KL11	0.69	KA45	0.57	KB08	0.80	KG04	0.61	IG14	0.69	KA07	0.53
KJ12	0.82	KL03	0.69	RG05	0.57	MY14	0.79	KL07	0.60	RG15	0.69	RK18	0.52
KL14	0.82	HM10D	0.68	BS09D	0.56	MR06	0.79	KM05	0.60	BU13	0.68	KJ17D	0.52
KI05	0.81	BG10	0.67	JJ05	0.56	RK01	0.78	MK14G	0.60	KM04C	0.68	RK14	0.52
JJ13	0.81	RG07	0.67	RG18	0.56	KI21	0.78	KG18	0.58	HM12	0.68	MA08	0.51
BG13	0.81	KG03	0.67	KL04	0.56	HM06	0.77	KZ08	0.58	KA49	0.68	KY15G	0.50
KJ13	0.80	KB12	0.67	KA08	0.56	KA53	0.77	MA13	0.58	KM08	0.67	KL06E	0.49
BG11	0.80	KG12	0.66	MK06	0.56	KI15	0.77	KA39	0.58	RK08	0.67	JJ15E	0.49
KL12	0.79	BS03	0.66	MS08D	0.56	JJ16E	0.74	JJ17	0.57	KW25	0.67	BS10	0.49
KL05	0.78	RG17	0.66	BS19	0.55	MK07F	0.73	HM11	0.57	KG01	0.66	MK05	0.48
KW01	0.78	BU08	0.66	JJ13	0.55	KJ01a	0.73	KM20	0.57	KA37	0.65	MA12G	0.48
BG14	0.78	HM01	0.65	RG01	0.55	KA46	0.73	KZ13	0.57	KW21	0.65	KB18	0.47
MY09	0.78	MR18	0.65	MY03	0.55	KZ10	0.73	BG12	0.56	BU01	0.63	KW13	0.46
KJ03	0.78	BS08	0.64	KM05	0.55	RG09	0.72	MA09	0.55	KB13	0.62	KM03A	0.45
MY11	0.76	BG14	0.64	KJ06	0.54	KZ02	0.72	BG05	0.55	KG09	0.61	KM07	0.45
BG02	0.75	KG08	0.64	KM02B	0.54	KG15E	0.70	HM18	0.55	HM03	0.61	KJ02	0.45
KW07	0.75	KZ06	0.63	KY12	0.54	MK04	0.70	KG21	0.54	KA44	0.60	RK03	0.44

Appendix 19: Shows ten important primary attributes used to evaluate cultivated Robusta coffee flavour in roasted ground coffee and brew

Attribute	What was assessed	Time evaluated	Score scale	How attribute was assessed
1.Fragrance or aroma Dry roasted ground coffee Wet or brewed ground roasted coffee	Type and Intensity of dry fragrance Type and Intensity of dry fragrance	Within 15 minutes of grinding 3-5 minutes after infusion with boiled water	Wet and dry smell rated 1-6 separately but finally combined to 1-10 score.	Sniffing roasted dry ground coffee powder Gently sniffing broken crust as you stir thrice and as the coffee steeps
2. Flavour	Intensity, quality and complexity of the taste aroma	8-10 minutes from infusion (about 160^{o}F or 70^{o}C), a temperature at which the retro nasal vapours are at maximum intensity.	1-10	Vigorously slumming coffee brew into the mouth while aspirating the liquor to cover much of the tongue area and upper palate to give a combined impression of gustatory (taste bud) sensations and retro nasal aromas that go from the mouth to nose.
3. Aftertaste (brackish or savory)	Lingering remnant sensation experienced at the back of the throat after swallowing and its changes over time.	As above	1-10	As above. Lower scores were given for short and unpleasant aftertaste.
4. Mouth feel (rough, smooth)	Liquor weight and texture	160 ^{o}F-140 ^{o}F	Both weight and texture rated 1-6 separately but finally combined to 1-10 score.	Comparing the viscosity [weight and slipperiness (texture)] of coffee liquor on the tongue with that of pure water
5. Bitter/Sweet Aspect Ratio (bitter sweet, low bitter, high sweet)	Available sweetness and bitterness	160 ^{o}F-140 ^{o}F	Bitter and sweet attributes rated 1-6 separately but finally combined to 1-10 score	Comparing relative balance of bitter and sweet taste. More marks for sweeter taste
6. Balance	Combined intensities of flavour, aftertaste, mouth feel and bitter/sweet	160 ^{o}F-140 ^{o}F	1-10	Comparing how flavour, aftertaste, mouth feel and bitter/sweet aspect ratio synergies combine and best achieved. When the four occur

	aspect ratio.			in equal intensities.
7. Softness	Liquor acidity and sweetness	Below 100 ^{o}F	1-10	Assessing perceived liquor acidity and sweetness as not masked by high salty-bitterness
8. Uniform cups	Flavour consistency or negative attributes in the cup	Below 100 ^{o}F	1-10	Evaluating flavour consistency or absence of any defect (sour, fermented, phenolic or other off taste) in the different cups brewed with same sample
9. Clean cups	Lack of defects or a non-coffee taste in a cup	Below 100 ^{o}F	1-10	Detecting absence of defect (moldy, dirty, baggy, off taste bean) in sample liquor in different cups right from ingestion to aftertaste
10. Overall score	Personal general assessment of the coffee brew.	70^{o}F or 16^{o}C	1-10	Combining all liquor flavor attributes in the coffee brew to a single personal judgment of one score guided by past experience
Note Attribute evaluation was done 2-3 while the liquor brew cooled Total scores were obtained by summing scores for the different primary attributes. The maximum score ranged from 1-100 Final score was not computed as it involved deducting scores for taints and faults from the total score. Based on the objective of the study, defects are correctable unless genotypic in nature or environmentally influenced.				

Appendix 20: Scale that was used to rate farmer Robusta coffee organoleptic cup

Total Score	Quality Description	Classification
90-100	Outstanding	Very Fine
80-90	Fine	Fine
70-80	Very good	Premium
60-0	Average	usual good quality
50-60	Fair	usual good quality
40-50	Fair	Commercial
<40		Exchange grade
<30		Below grade
<20		Off grade
<10		Triage

Appendix 21: Organoleptic cup attribute diversity groups accessions derived from K means analysis

Group 1		Group 2		Group 3		Group 4
BG05	RG08	BG11	KY05	BG14	MS08D	BG16
BS15	RG15	BG12	KY13	BS01	RG02	BS04
BU06	RG17	BG17	KY17C	BS18	RG03	BS11
BU12	RK01	BS06	KY20	BU01	RG06	BU04
HM04C	RK02	BS08	MA03	BU14	RG10	HM13
HM09	RK03	BS16	MA04	BU15	RK06G	JJ06
HM26	RK09G	BU05	MA05	HM07	RK13	JJ07
IG07	RK10	BU08	MA11	HM10D	RK14	JJ13
JJ03	RK15	BU10	MA15	HM12	RK17	KA16
JJ05	CN01	BU13	MK07F	IG14	RK18	KA42
KA31	CN03	HM06	MK12E	JJ01		KB03D
KA37		HM16	MR04	JJ02		KB09
KA52		HM17	MR12	JJ09		KB18
KB04G		HM18	MS11	JJ15E		KI10
KG01		HM22	MY08	KA12		KI16
KG03		IG04	RG01	KA22		KJ01a
KG04		IG13		KA29		KJ05
KG18		JJ08		KA36		KJ26
KI19		KA01G		KA43		KL08
KJ16G		KA03		KA47		KM19
KL03		KA30		KA49		KW02
KL10		KA39		KB11		KW06
KL12		KB02		KG02		KW15
KL15		KB17		KI02		KW22
KM08		KG05		KJ17D		KY11a
KM12		KG09		KL04		KY16
KM14		KG13D		KL18		KZ11
KM16		KG15E		KL20		KZ15
KM17		KG21		KM02B		KZ16
KM23		KI05		KM04C		MA08
KW19		KJ02		KM22		MA12G
KY12		KJ10		KW08		MA17
KZ12		KJ21		KW17		MK01
MA10		KJ23		KY03		MK05
MA16		KL01		KZ07		MK11
MA18		KM03A		KZ09		MR13
MK03		KM05		KZ14		MS07
MK04		KM21		KZ18		MY16
MK10		KW01		MA02		RG07
MR01D		KW03		MK08		RK05
MR19		KW04		MK13		RK16
MS10		KW07		MR10		CN02
MY01		KW11E		MR11		

Appendix 22: Farmer Robusta coffee accessions that had high organoleptic cup attribute variability with the principal component analysis (squared cosines of the observations).

F1								F2		F4	
Flavour, aftaste, salt/acid, mthfeel, bal								Fragrance aroma		Bitter sweet	
MK11	0.96	JJ06	0.77	KI16	0.60	KW02	0.47	BS01	0.90	RG10	0.88
KB03D	0.95	KG04	0.76	RG02	0.59	IG13	0.47	KM05	0.85	KG02	0.63
KB18	0.94	JJ05	0.75	KA31	0.59	KY11a	0.46	HM12	0.81	BS18	0.62
BG05	0.93	MS10	0.74	KJ26	0.59	RK02	0.46	KI05	0.79	RK17	0.60
KA42	0.92	KL15	0.74	MR13	0.59	RK01	0.44	KM22	0.69	MK08	0.59
JJ03	0.90	BS11	0.74	KM17	0.58	KY12	0.43	KZ07	0.69	BU13	0.55
KM19	0.89	BS04	0.73	KM08	0.58	MA08	0.41	KJ17D	0.68	KW03	0.53
MA17	0.89	KW15	0.72	KJ01a	0.58	KZ15	0.41	KZ14	0.67	HM22	0.49
MY01	0.88	MA10	0.71	RK05	0.58	KG21	0.40	KW04	0.66	KJ10	0.47
MA16	0.88	RG15	0.71	MK04	0.57	KL03	0.36	KW17	0.65	IG14	0.45
RK16	0.88	BS15	0.71	MS11	0.57	BU01	0.35	KI02	0.62	KA39	0.44
RG17	0.86	JJ07	0.70	RG07	0.57	MR04	0.34	KA29	0.62	MY08	0.43
IG07	0.86	BU15	0.70	KL12	0.57	HM17	0.33	KJ02	0.61	KL18	0.43
KW22	0.85	KJ05	0.68	MK01	0.57	KW11E	0.32	KG13D	0.59	MA02	0.41
MK05	0.85	KZ11	0.68	JJ15E	0.55	MK07F	0.31	KA30	0.58	HM06	0.40
MK10	0.85	KI10	0.68	MA04	0.54	MA18	0.29	BS16	0.57	RG03	0.40
KI19	0.84	KG01	0.67	KG18	0.54	MR19	0.27	KA43	0.54	RK15	0.40
KL08	0.82	KA16	0.67	KA37	0.54	MR10	0.26	RK13	0.49	KB02	0.39
HM04C	0.81	KW06	0.67	KL01	0.53	KY03	0.26	BG17	0.49	JJ02	0.38
KZ16	0.81	MS07	0.65	RK18	0.52			KL20	0.46	KG05	0.37
HM09	0.81	KW19	0.65	RK10	0.51			BU10	0.45	KJ23	0.37
MR01D	0.80	KY16	0.65	BG16	0.50			RK03	0.42	RG01	0.35
KM12	0.80	KL10	0.65	HM13	0.49			KY17C	0.41	KG03	0.32
JJ13	0.79	HM26	0.63	MK03	0.48			MA03	0.36	KM21	0.31
MA12G	0.79	KM23	0.63	RK09G	0.48			KM04C	0.36	KA47	0.25
KM16	0.78	KZ12	0.61	KA52	0.48			KM02B	0.32	HM10D	0.24
MY16	0.78	RG08	0.61	BU04	0.48			KJ21	0.32		
BU06	0.78	KJ16G	0.60	KB09	0.48			KW07	0.31		

Appendix 23: Diversity of farmer Robusta coffee flavours in fresh ripe cherry pulp, green and roasted beans

Fresh ripe cherry pulp	Dry raw beans	Roasted and brewed ground roasted beans	
		Fine notes	Commercial notes
Blackberry, apple banana, guava, lemon, honey, apple, fermented, stinky insect, ripe mango, ginger, lime, milk chocolate, rotten egg	Clean or nutty smell	Enzymatic (honey, lemon) fruitlike (cherry, black current, raisin, berry, raspberry, dry fig, & prunes), nut like (almond), spice like (clove, coriander spice), sweet like (molasses, syrup, dark chocolate); malt; sugar Browning notes (buttery, vanilla, caramel, cocoa, walnut, <u>Additional brew assessment remarks</u> Rounded, complex, complete, mellow, deep and delicate taste	Vegetable like (citrus taste, buttery, herbal, grassy, peas, potato, cereal, eucalyptus, phenol like (bitter, honey, carbon, tar like, rubber, boiled meat); astringent (brackish, salty) weak liquor, low flavor, slight fruity flavor <u>Additional brew assessment remarks</u> dull, lifeless, flat, uneven, neutral, harsh and soapy taste

Note
Fine notes (brew that can attain specialty grade); Commercial notes (brew of average category)